USDA FOREST SERVICE, NORTHERN REGION

ENVIRONMENTAL ANALYSIS REPORT

PILOT PROJECT TO EVALUATE DYLOX AND ORTHENE FOR CONTROLLING THE WESTERN SPRUCE BUDWORM

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Submitted Thomas H. Flavell 3/17/76
THOMAS FLAVELL
Project Director, FEP

Concur James R Jordan
JAMES JORDAN
Forest Supervisor, Helena NF

Approved LeRoy Jones
LEROY JONES
Deputy Regional Forester, S&PF

f. Impact of fires and bugs on long-term maintenance of habitat.

g. Aquatic monitoring, [illegible] National Forest System lands.

h. Reforestation trust.

i. Project work plus.

PILOT PROJECT TO EVALUATE DYLOX^{R} AND ORTHENE^{R} FOR CONTROLLING THE WESTERN SPRUCE BUDWORM

CONTENTS

I. SUMMARY

The Forest Service proposes a pilot control project on 6,000 to 11,000 acres on the Helena National Forest in June and July 1976, to determine the effectiveness of Dylox and Orthene on the western spruce budworm.

Both Dylox and Orthene cause mortality to some beneficial insects. Orthene is highly toxic to honey bees. The project will have a favorable impact on the local economy for the summer. No long-term biological impacts from the project are expected.

Malathion is currently the only available insecticide registered for control of the western spruce budworm. It is a broad-spectrum insecticide registered for more than 100 economic insect pests. A no-action alternative would leave the Forest in the current position of having no alternatives to Malathion for chemical control of the budworm.

This project is part of a Nation-wide effort to develop environmentally acceptable chemicals for control of important forest insects. A pilot control project is required by the Forest Service and considered desirable by the Environmental Protection Agency before any chemical can be registered for insect control.

II. DESCRIPTION

There is a need for the development of effective, environmentally acceptable control measures for the western spruce budworm (*Choristoneura occidentalis* Freeman). Biology of the insect and its impacts on the forest are given in detail in Appendix A. The development and recommendations for use of any pesticide by the Forest Service follow an established program. The materials are first tested in the laboratory under a variety of bioassay procedures. Materials found to be promising candidates in the laboratory are then evaluated in small-scale field tests which usually test several formulations and application rates. Residue determinations and environmental monitoring are initiated. The next step is the pilot control project where the most promising formulation and application rate are evaluated under operational conditions. Recommendations to evaluate two of these materials, Dylox^{R} (trichlorfon) and Orthene^{R} (acephate) in the Northern Region emerged from a work planning session held in Washington, D.C., on October 15-16, 1975. This is part of a Nation-wide effort to meet the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act of 1972, as amended, for registration of pesticides for specific pests. Supporting insecticide research and development programs are detailed in Appendix B. In developing this environmental analysis, an interdisciplinary team consisting of these specialists was consulted: entomologist, pesticide specialist, wildlife biologist, fisheries biologist, plant pathologist, forester, forest line officer, and information specialist.

Proposed Action

The Forest Service proposes a pilot control project on six treatment blocks on 6,000 to 11,000 acres on the Helena National Forest in June and July 1976, to determine the effectiveness of Dylox and Orthene on the western spruce budworm. Rates of application per acre for the project are:

Dylox--One pound of active ingredient in enough Panasol^{R} to make ½ gallon of spray.

Orthene--One pound of active ingredient in enough water to make 1 gallon of spray.

Background data on Dylox is given in Appendix C; background data on Orthene is given in Appendix D.

Objectives

The objectives of the pilot control project are:

1. To evaluate the effectiveness of Dylox and Orthene as control agents for the western spruce budworm when used under operational conditions.

2. To evaluate the level of current year foliage protection.

3. To identify and resolve formulation, application, and safety problems associated with the use of these pesticides on an operational scale.

4. To evaluate the impact of these pesticides on aquatic invertebrates and fish when used operationally.

5. To assess the impact of Orthene on parasites of the western spruce budworm.

A supplemental objective, based on available financing, is to evaluate the effects of spraying Orthene on the population dynamics of the western spruce budworm.

Location

The proposed site for this pilot project includes portions of the Belt Mountain range on both the Townsend and Canyon Ferry Ranger Districts on the Helena National Forest, Montana.

Defoliation by the spruce budworm has been evident over extensive areas on this Forest since 1974. A general survey for budworm defoliation in 1975 estimated a total of 473,937 acres were affected on the Helena National Forest; up 45.2% from 1974 (Tunnock et al., 1976). A followup egg mass survey to predict defoliation on the Forest indicated damage will probably be higher in 1976.

A detailed egg mass survey was made in December 1975 to evaluate population densities in the Douglas-fir type on the west slope of the Big Belt Mountain range for possible use in a pilot project. The mean new egg mass density in these drainages is as follows:

	Drainage	Egg masses/1000 in^2	Predicted defoliation
1.	Dry Creek (lower sec.)	6.3	20%
2.	Deep Creek (E. Fork)	16.1	45%
3.	Deep Cr. (Middle & W. Frk)	17.7	45%
4.	Sulphur Bar Cr.	25.7	60%
5.	Holloway Gulch	9.8	30%
6.	Confederate Gulch	10.8	30%
7.	White Creek	18.4	50%
8.	Culp Gulch	13.0	35%
9.	Never Sweat	13.4	35%
10.	Indian Creek	11.2	30%
	Mean =	14.2	Mean expected defoliation 45%

Effectiveness of Proposed Materials Against Target Insect

Orthene.--After preliminary laboratory tests by the Pacific Southwest Forest and Range Experiment Station, Forest Insect and Disease Management in the Northwest Region, USDA Forest Service, conducted a cooperative field experiment in 1975 with the Pacific Southwest Forest and Range Experiment Station using Orthene. Three 20-acre plots were treated with 1 pound of Orthene in 1 gallon of water per acre applied early (at 50 percent budbreak). The same treatment was applied to three additional 20-acre plots later when most of the insects were out of the buds (50 percent of larvae in fifth instar). Three untreated plots were used as checks. Applications were made by helicopter. Results of the field experiment are summarized in Table 1.

Table 1.--Effectiveness of Orthene on western spruce budworm in Washington in 1975.

Treatment	Average larvae per 100 buds: Prespray	Average larvae per 100 buds: Postspray 14 days	Budworm mortality percent	Defoliation percent
Early Orthene	37.0	17.1	53.8	21.6 a/
Early untreated	42.4	38.0	10.0	64.0 a/
Late Orthene	27.2	0.1	99.6	96.9 b/
Late untreated	43.0	20.2	53.0	100.0 b/

a/ The amount of defoliation present at the 14-day postspray sample taken during first week of July.

b/ The amount of defoliation present at the 14-day postspray sample taken during last week of July.

Dylox.--The effectiveness of Dylox on eastern spruce budworm has been summarized by Kettela (1974) from 1970 and 1973 field tests in New Brunswick. The 1970 tests were two applications of Dylox in an oil carrier, and the 1973 tests were two ultra low volume (ULV) applications of Dylox only. Results of these field tests are given in Table 2.

Randall (1970) achieved good results when Dylox was sprayed at the peak of the fifth and sixth instar, and apparently good foliage protection was achieved by spraying at the peak of the third instar. Randall's data is summarized in Table 3.

Forest Insect and Disease Management in the Western Region, Forest Service, conducted a pilot control program of Dylox on Modoc budworm, *Choristoneura virides*, in northern California. Results of this pilot control program are given in Tables 4 and 5.

Table 2.--Effectiveness of Dylox on spruce budworms sprayed at peak fifth and sixth instar of larval development. (Kettela, 1974).

Treatment	Year	Tree species	Insects per 18-inch branch tip: Pre-spray L3 a/	Postspray L5	Postspray L6	Postspray Pupa	Survival Pupa	Defoliation percent Obs.	Defoliation percent Exp.	Percent foliage saved
6 oz + 6 oz each in 0.5 gal./acre	1970	fir	7.83	3.60	1.60	0.11	1.4%	20	40	50
Control	1970	fir	8.50	-	-	1.58	19.0%	40	-	-
2.3 + 6 oz/acre in 0.15 gal.	1973	fir	13.40	-	1.87	1.36	10.0%	22	56	61
Control	1973	fir	25.00	-	12.20	10.20	41.0%	62	-	-
6+6 oz.	1970	spruce	3.40	1.88	.88	.04	1.1%	-	-	-
Control	1970	spruce	4.55	-	-	1.03	23.0%	-	-	-
2.3+6 oz.	1973	spruce	17.60	-	1.33	.63	3.5%	-	-	-
Control	1973	spruce	19.30	-	1.90	1.80	9.3%	-	-	-

a/ L3 etc. - denotes larval instar at time of sampling
- - denotes no sample or estimate or not applicable

Table 3.--Effect of Dylox on spruce budworm (Randall 1970)

Trials conducted in 1970 in the Scoudouc area of New Brunswick

Block	Date sprayed	Insect development	Dosage (oz/acre)	Average % reduction Fir	Average % reduction Spruce	Percent defoliation Fir	Percent defoliation Spruce
1	29 May	63% 3rds	5.6	29	7	5	5
	4 June	4ths	5.6				
14	17 June		5.6				
	19 June	37% 6ths	5.6	95	89	10	13
Control	--	--	--	--	--	98	97

Table 4.--Effectiveness of Dylox on Modoc budworm, 6-day postspray sample--population reduction (Forest Insect and Disease Management, Western Region).

Population	Treatment (Dylox applied per acre) 1 pound	3/4 pound	Zero
High	97.80	89.81	58.98
Medium	97.67	89.30	57.46
Low	94.45	87.96	66.64

Table 5.--Percent control of Modoc budworm treated with Dylox 6-day postspray sample (Forest Insect and Disease Management, Western Region).

Population	Treatment = Dylox applied per acre 1 pound	3/4 pound
High	90.20 ± 11.13	59.93 ± 17.55
Medium	96.04 ± 6.01	69.69 ± 20.29
Low	80.45 + 17.96*	75.62 ± 22.23
Average for treatment	86.90 ± 6.33	68.38 ± 8.96

*Possibly influenced by rainstorm in the evening of spray day.

The Northern Region, Forest Service, conducted a pilot control program of Dylox on the western spruce budworm in central Montana in 1975. Treatments were 1 pound of Dylox plus enough oil carrier to make 1 gallon of spray per acre and untreated checks. Results of this pilot control program are given in Table 6. Dylox is being tested in the Northern Region again at a different formulation and a different application strategy. This is based on promising results using a formulation of 1 part Dylox to 1 part carrier on the eastern spruce budworm.

Table 6.--Effectiveness of Dylox on western spruce budworm in central Montana.

		14-day postspray		21-day postspray	
Treatment	Population[a/]	Population[a/]	Percent control[b/]	Population[a/]	Percent control[b/]
Check	19.36	19.34	--	16.51	--
Dylox	18.80	6.01	68.49	3.72	76.88[c/]

a/ Average number larvae per 100 buds.
b/ Mortality attributed to pesticide.
c/ Significant at the 0.01 level.

Both chemicals are now ready for the third step in registration, the pilot project which is in this proposal.

III. ENVIRONMENTAL IMPACTS

Physical and Biological Impacts

Dylox

A. Mammals, Birds, Fish

The low order of oral and dermal toxicity of Dylox to mammals permits it to be used as treatment for the control of internal and external parasites of livestock. Dylox administered to 200 calves at rates of up to 5 grams per 100 pounds of body weight did not cause death (Andrews and Dugar, 1972). Dylox is used on rangeland without restrictions on grazing or the removal of livestock from the treated area at time of spray application (Chemagro, 1973).

Dylox should not have an adverse effect on birds present in the treatment area if applied correctly. Effect of Dylox on nesting birds was studied by DeWeese and Henny (1976) on the Beaverhead National Forest during a pilot control project in 1975. Breeding bird density and diversity showed similar patterns on check and treated plots after treatment. Searches for sick or dead birds showed no increase in mortalities on treated plots. Zinkel et al. (1976) studied brain cholinesterase depres-

sion 1/ of birds on the same pilot control project. Only minimal exposure occurred at the rate of Dylox application (1 pound per acre), and the spray had little effect on brain cholinesterase activities. These two reports are attached as Appendices E and F.

Haugen (personal communication) monitored two streams on the Beaverhead National Forest purposely sprayed with Dylox during a pilot control project in 1975. Dylox caused a measurable increase in drift 2/ of mayflies (Ephemeroptera), stoneflies (Plecoptera), and true flies (Diptera) one-half to 2 hours after application to one of the streams; no increase in drift was measured in the second stream. Trout placed in live cages in the streams 1 to 2 weeks prior to treatment and removed for residue analysis showed no physical disability as a result of the application. Residue analysis of fish tissue showed 0.03 ppm (parts per million) Dylox.

B. Bees and Other Beneficial Insects

Several years of use of Dylox on a wide variety of crops have shown it to be relatively nontoxic to honey bees and other pollinators (Chemagro 1973, 1975). Direct aerial application of Dylox over hives showed that the chemical had no harmful effects on behavior, mortality, or honey yield (Andrews and Dugar, 1972). No special precautions or removal of bee hives is necessary in areas to be treated with Dylox.

In tests on alfalfa and cotton, application of Dylox at 1 pound per acre caused substantial reductions in populations of beneficial insects such as big-eyed bugs, damsel bugs, and lady beetles (Andrews and Dugar, 1972). Populations of these insects returned to pretreatment levels within 1 to 2 weeks following application.

C. Plants, Soil, Water

Chemagro (1973, 1975) reports that Dylox applied at 10 ppm rapidly disappears in the soil and there is no buildup of Dylox residues from one growing season to the next. In another study (Andrews and Dugar, 1972), Dylox was sprayed on three soil types: sandy loam, silt loam, and high organic silt loam. Application was made at 20 pounds per acre followed by simulated rainfall applied once weekly for 5 weeks. After the 5-week period, residues in the runoff water, as a percentage of the total applied, were silt loam, 2.86 percent; sandy loam, 0.65 percent; high organic silt loam, 0.35 percent (Andrews and Dugar, 1972).

1/ Cholinesterase is a key enzyme of the nervous system which is essential for normal nerve function. Depression of cholinesterase allows constant transmission of nerve impulses, and if continued long enough, results in death.

2/ The normal flow of aquatic insects with the current in a stream is called drift. An increase in drift is an increase in insect numbers flowing with the current after application of an insecticide.

Residue analysis in New York 26 days following application of Dylox at 1 pound per acre showed 0.33 to 3.3 ppm on leaves, 1.1 ppm on twigs, and 1.5 ppm on the forest litter (Wilcox, 1971).

The half-life 3/ of Dylox in water in the dark at 30°C at pH 5 was 4.7 days; at pH 7, 0.6 days; and at pH 9, 0.1 days (Chemagro, 1975). Dylox in an outdoor pond (pH 7 and temperature 29°C average) with exposure to sunlight and wind showed a half-life of only 0.3 days.

Based on a careful review of research results in the References Cited section of the Appendix, the Dylox applied in this project will have no effect on mammals, birds, fish, plants, soil, or water. It will be harmful to some beneficial insects, and may cause increased drift of aquatic insects when applied directly to streams.

Orthene

A. Mammals, Birds, Fish

Feeding studies show that Orthene is rapidly eliminated by mammals and birds and is not retained in body tissues (Chevron Chemical Company, 1975). Orthene is eliminated in the cow and goat mainly in the urine, with the remainder in the feces and milk. The main route of excretion in quail and chicken is through the feces with the remainder in the eggs. Orthene does not have long-term toxic effects and does not significantly affect reproduction or the health of succeeding generations.

Studies were conducted to determine Orthene's ability to irritate eyes and skin in mammals (Chevron Chemical Company, 1975). Direct contact with Orthene was irritating to the eyes of rabbits, but eyes appeared normal 14 days after exposure. Contact with Orthene caused moderate skin irritation on abraded skin of rabbits, but no irritation on intact skin.

Fish (bluegill, yellow perch, smallmouth bass, and bullhead) continuously exposed to Orthene accumulate small quantities of the chemical in body tissue (Chevron Chemical Company, 1975), but Orthene is rapidly eliminated as levels of the chemical in the water drop; after 4 days, Orthene residue could not be detected (LOTEL, 1975). Since Orthene's toxicity to fish is extremely low and it is rapidly degraded under natural conditions, it poses little hazard to fish populations (Shea 1975).

B. Bees and Other Beneficial Insects

Orthene is highly toxic to honey bees directly exposed to it, and application when honey bees are active should be avoided (Chevron Chemical Company, 1973, 1975).

3/ Half-life is the length of time required for one-half of the material to break down to the point where it can no longer be detected.

In tests to determine its effects on other beneficial insects (Chevron Chemical Company, 1973, 1975), Orthene was virtually inactive against the wasp, *Chelonus blackburni*, and the lacewing, *Chrysopa carnea*. It was less toxic than parathion or diazinon on the ladybug, *Hippodamia convergamis*, and the wasp, *Muscidifurax raptor*. Orthene was about as toxic as parathion to the hyperparasitic wasp, *Tachinaephagus zelandicus*. Orthene will reduce populations of some beneficial insects present at the time of application.

C. Plants Soil, Water

When Orthene is applied, it is rapidly degraded by plants and soil and more slowly by water (LOTEL, 1975; Chevron Chemical Company, 1975).

Orthene penetrates plant tissues quickly - 80 percent is absorbed within 24 hours. Orthene has a half-life in plant tissue ranging from about 5 to 10 days (Chevron Chemical Company, 1973, 1975).

Soil microorganisms rapidly degrade Orthene so that it is not available to succeeding crops (Chevron Chemical Company, 1973, 1975). In laboratory tests with 9 soil types, half-life ranged from one-half to 4 days in 8 of the soil types tested, and 6 to 13 days in muck soil.

Orthene breaks down relatively slowly in water, and the rate of hydrolysis is affected by temperature and alkalinity. Under laboratory conditions, the half-life of Orthene in water at pH 7 and 21°C is 46.4 days (Chevron Chemical Company, 1975). In natural bodies of water, degradation is accelerated by breakdown in submerged vegetation and soil microorganisms in bottom mud, and there is little risk of contaminating water supplies (LOTEL, 1975).

Based on a careful review of research results in the References Cited section of the Appendix, the Orthene applied in this project will have no long-term effects on mammals, birds, fish, plants, soil, or water. It will be harmful to some beneficial insects. Orthene is extremely toxic to honey bees and will kill those present on the spray blocks during application.

Dyes.--Dyes will be added to the spray solutions for spray deposit analysis. Automate Red B^{R} will be added to the Dylox spray to make up 2 percent of the spray volume. Rhodamine B extra S^{R} will be added at a rate of 1.198 g per liter (1 lb/100 gal) to the Orthene formulation. The impact of these dyes on the environment is unknown, but is not expected to be significant.

Panasol AN-3^{R}.--Panasol is the oil carrier that will be used for the Dylox spray. Panasol has been tested on Burley tobacco, cotton, and corn with only slight injury (Amoco Chemicals Corporation, 1962). These three plants are normally used in solvent tests. Panasol has not been tested on forest vegetation, but it is not expected to cause any phytotoxicity. Panasol may cause spotting of paint on vehicles.

Helicopter noise.--The spray ship will cause enough noise to disturb birds and animals on the spray areas. This may cause them to leave the area temporarily, but no permanent migration out of the spray blocks is expected.

Other physical and biological impacts.--There are no known threatened or endangered plant or animal species present on any of the proposed spray blocks. There will be no impact on historical or archeological features, wilderness characteristics, or any other resources as a result of the project.

Social and Economic Impacts

Dye or carrier may damage clothing and vehicles if anyone is present during spraying. Orthene also has a disagreeable odor, which is offensive to many people.

The project will be temporarily beneficial to the economy of Townsend for the summer. Some local people will be employed. People employed from other areas will be purchasing lodging, food, fuel, and probably clothing.

A benefit-cost analysis of a pilot control project is not normally made for these reasons:

1. The pilot control project cost records form the basis for benefit-cost analyses of future operational control projects.

2. A pilot control project is conducted to determine effectiveness of a control method, not to control an outbreak.

IV. ADVERSE ENVIRONMENTAL EFFECTS WHICH CANNOT BE AVOIDED

Dylox.--Dylox may cause increased drift of aquatic insects when applied directly to streams.

Orthene.--Orthene will reduce populations of some beneficial insects such as the lady beetles and some parasites and predators. Any honey bees present on the treatment area will be killed.

Helicopter noise.--The noise from the helicopters cannot be avoided.

V. RELATIONSHIP BETWEEN LOCAL SHORT-TERM USES OF MAN'S ENVIRONMENT AND THE MAINTENANCE AND ENHANCEMENT OF LONG-TERM PRODUCTIVITY

There are no known long-term effects of the project on productivity since this is not an operational budworm control project. If one or both of the chemicals are registered by EPA, their future use could have a long-term effect on productivity. Both favorable and adverse impacts will be covered in an appropriate environmental analysis report or environmental statement prior to any operational projects.

VI. IRREVERSIBLE OR IRRETRIEVABLE COMMITMENT OF RESOURCES

There will be no known irreversible or irretrievable commitment of threatened or endangered plant or animal species, historical or archeological features, wilderness characteristics, or any other resources as a result of the proposed action.

VII. ALTERNATIVES TO PROPOSED ACTION

No action.--Malathion is currently the only available insecticide registered for control of the western spruce budworm. This course of action would leave the Forest Service in the current position of having no alternatives to Malathion for chemical control of the budworm.

This project is part of a nationwide effort to develop environmentally acceptable chemicals for control of the western spruce budworm. A pilot control project is required by the Forest Service and considered desirable by the Environmental Protection Agency before any chemical can be registered for insect control.

In an operational program, other alternatives that would be considered are other chemicals, parasites, predators, and biological insecticides.

VIII. CONSULTATION WITH APPROPRIATE FEDERAL AGENCIES AND REVIEW BY STATE AND LOCAL AGENCIES DEVELOPING AND ENFORCING ENVIRONMENTAL STANDARDS

This analysis was prepared and reviewed by an interdisciplinary Forest Service team. In addition, the list of References Cited and the articles in the Appendix were consulted in writing this analysis.

IX. MANAGEMENT REQUIREMENTS AND CONSTRAINTS

1. Spray aircraft will be routed where possible so as not to fly over improved agricultural land.

2. No permits for domestic honey bee colonies will be issued for the treatment areas during the spray period.

3. All honey bee owners within 3 miles of the spray blocks will be notified of the project.

4. All nonproject persons and vehicles will be kept out of the treatment blocks during spraying because of possible damage to clothing or vehicle paint.

Operating instructions are spelled out in greater detail in the project work plan.

X. ENVIRONMENTAL STATEMENT RECOMMENDATION

This is not a major Federal action and will result in no major environmental effects; therefore, an environmental impact statement will not be written.

XI. APPENDIX

A. Biology of the western spruce budworm and its impacts on the forest.

B. Supporting research and development programs on insecticides.

C. Background document for ᴿDylox insecticide.

D. The impact of Orthene on the environment.

E. The impact of Dylox and Sevin on breeding bird numbers and nesting success.

F. The impact of Dylox and Sevin on brain cholinesterase activity in birds.

G. Aquatic monitoring, 1975 Beaverhead National Forest Dylox test.

H. References cited.

I. Project work plan.

APPENDIX A: Biology of the western spruce budworm and its impacts on the forest.

Western Spruce Budworm Biology
(Based on Carolin and Honing, 1972)

Description.--Adult moths are about 12 mm. long and have a wingspread of 22 to 28 mm. The gray-brown or orange-brown forewings are marked with bands or streaks, and each usually has a conspicuous white dot on the margin of the wing. Eggs are oval, light green, and about 1.2 mm. long. They are deposited in masses on the undersides of the needles, with individual eggs overlapping like shingles. Usually there are six larval instars. Newly hatched larvae are green with brown heads. During the second to fourth instars, the larvae have black heads and collars and an orange-brown or cinnamon-brown body. In the fifth instar, the larvae have reddish-brown heads marked with black triangles, a black collar, and a pale olive-brown body marked with small, whitish spots. Full-grown larvae are 25 to 32 mm long, with tan or light chestnut-brown heads and collars, and with large ivory-colored areas superimposed on an olive-brown or reddish-brown body. Pupae are 12 to 15 mm long, broad at the head end but tapering rapidly toward the tail. They are brownish-yellow or occasionally brownish-green when first formed and later turn reddish-brown.

Life history.--The budworm normally develops from egg to adult in 12 months, but some may require 24 months. Adult moths emerge from pupal cases in late July or early August. Shortly afterward the females deposit egg masses. Each female lays approximately 150 eggs; egg masses average 25 to 40 eggs. The eggs hatch in about 10 days. The newly hatched larvae do not feed but seek hiding places among lichens or under bark scales on the host trees. They spin silken shelters (hibernacula) and remain dormant through the winter. In the spring, larvae move to foliage, tunnel into needles, and feed for 7 to 14 days. When buds start to swell, larvae leave the needles and bore into expanding buds. They sometimes move directly from hibernation to vegetative buds or male and female flowers. As new shoots unfurl, the larvae spin loose webs between needles and tips. They feed on new foliage within the webs. New foliage is completely destroyed before they feed on older needles. Larvae become full grown in 30 to 40 days after attacking buds and pupate either in existing webs or webs they spin other places on the tree. Adult moths are sluggish fliers but may be carried great distances by air currents, thereby spreading the infestation. The female deposits eggs within 7 to 10 days after emergence and dies. Large larvae usually feed on Douglas-fir and true fir foliage, even though their earlier feeding may have included staminate flowers and conelets. In the Northern Rocky Mountains, large larvae often feed on cones and seeds of western larch and Douglas-fir and pupate in cones.

Natural regulating factors.--Budworm populations normally are held in check by combinations of several natural control factors, such as parasites, predators, and adverse climatic conditions. However, when climatic conditions are favorable for an increase in budworm populations (decreased

numbers of low pressure centers, early and dry growing seasons), the combined effect of other natural factors cannot be relied upon to prevent an outbreak. During prolonged outbreaks, starvation can be an important factor in controlling budworm populations. Approximately 40 species of primary parasites (small wasps and flies) have been found attacking the budworm, with some 10 to 12 species exerting the most control. Spiders, ants, snakeflies, true bugs, and larvae of certain beetles are important predators of the budworm. Warblers, thrushes, sparrows, cedar waxwings, and evening grosbeaks are the more important birds feeding on the budworm. Budworm mortality from disease has been very low, even though the budworm has been found infected by several pathogens. Climatic conditions may adversely effect the budworm in several ways. Cool summer weather retards feeding and insect development; occasionally budworm eggs fail to hatch before the onset of freezing temperatures. Extreme temperature changes presumably have a detrimental effect on hibernating larvae, and sudden freezing temperatures in spring may kill larvae in needles, buds, or new shoots. Windy conditions at the time larvae hatch from eggs or are leaving hibernation quarters may disperse these larvae over wide areas. Prevailing winds and frontal disturbances can also disperse moths over a wide area.

Impact of Defoliation on Trees

Larvae of the spruce budworm complex are some of the most destructive defoliators of coniferous forests of North America. Freeman (1967) recognizes the western spruce budworm as separate and distinct from the eastern form; this western species is an important factor in the management of Douglas-fir and true firs in the Rocky Mountains and Pacific Northwest.

Repeated defoliation by budworm causes loss of radial increment (Williams, 1963, 1966, 1967), top kill (Silver, 1960), and tree mortality. Radial increment in heavily defoliated trees in Oregon was reduced more than 41 percent in grand fir but only 13 percent in Douglas-fir. Radial increment of Douglas-fir increased during the later stages of the outbreak, but that of grand fir and Engelmann spruce was still declining (Williams, 1963, 1966, 1967). In an infestation in British Columbia, many Douglas-fir had all their buds killed and lost over 90 percent of their foliage, but no trees on study plots were killed. Top and branch kill was common, but heavy adventitious budding made recovery rapid (Silver, 1960). Williams (1963, 1966), however, did not observe top killing of Douglas-fir in Oregon. Defoliation has a severe impact on understory regeneration which has less foliage area than larger trees and constantly intercepts larvae dropping from overstory foliage (Ghent, 1958). Mortality of understory regeneration generally occurs 2 to 3 years before permanent damage is noticeable in mature trees (Ghent, 1958).

Western spruce budworm is the most important insect enemy of Douglas-fir cones in Montana (Dewey, 1969, 1970, 1972). Heavy infestations in cones have resulted in total failures of seed crops, resulting in a lack of natural Douglas-fir regeneration in areas which have suffered mortality from budworm defoliation (Dewey, 1969, 1972).

Brief History of Past Epidemics and Suppression Activities

History of western spruce budworm outbreak and suppression activities in the northern Rocky Mountain and Intermountain States is described by Johnson and Denton (1975).

Annual reports of forest insect conditions were furnished by National Forest Ranger Districts from 1925 to 1953. These reports revealed that western spruce budworm was active in northern Idaho and Montana during most of the reporting period. The Helena National Forest had active infestations during this period.

The insect's potential as a forest pest prompted the Northern Region to inaugurate systematic annual surveys in 1950. Reports based on these surveys disclosed that outbreaks occurred on the Helena National Forest from 1950 to 1956 and 1960 to 1971. Acreage infested declined from 1966 to 1971, but has recently increased.

Suppression activities on the Helena National Forest since 1953 were:

1. Operational use of DDT at 1 pound per acre on 117,140 acres in 1953.

2. Operational use of DDT at 1 pound per acre on 153,630 acres in 1956.

3. Experimental use of DDT at 1 pound per acre plus Genite at 0.5 and 1 pound per acre on 18,200 acres in 1958.

4. Operational use of DDT at 0.5 pound per acre on 209,810 acres in 1962.

5. Operational use of DDT at 0.5 pound per acre on 35,130 acres in 1963.

6. Experimental use of Malathion at 12 fluid ounces per acre on 26,290 acres in 1964.

1976 Predictions

Aerial surveys conducted in 1975 revelaed budworm defoliation at 3,232,553 acres on six National Forests in Montana (Tunnock et al., 1976). Defoliated acreage on the Helena National Forest increased from 259,752 acres in 1974 to 473,937 acres in 1975, an increase of 45 percent (Tunnock et al., 1976).

Egg mass surveys in the fall of 1975 indicate that significant defoliation will occur in 1976 on the Helena National Forest. Predicted defoliation ranged from 5 to 79 percent (average 57) (Tunnock et al., 1976). A continuation of the infestation could disrupt timber productivity, have an impact on recreation esthetic values, and increase fire hazard in heavily defoliated stands.

The impact of western spruce budworm defoliation is difficult to quantify, although some attempts have been made. Bousfield et al. (1973) found a total net growth loss of nearly 20 board feet per acre per year between 1967 and 1972 in western Montana. Franc et al. (1973) showed a net decline in growth rate of 27.5 percent in northern Idaho. Ciesla et al. (1973) found up to 47 percent top kill of grand fir volume on limited areas of northern Idaho.

Impacts of western spruce budworm outbreaks on forest resources have been summarized by Johnson and Denton (1975).

APPENDIX B: Supporting research and development programs on insecticides.

The problem of budworm outbreaks can eventually be solved by an integrated pest management strategy designed to maintain populations below the level where measurable growth loss occurs. Strategies might include a combination of methods involving forest management practices, continuous population surveillance for damage and trend prediction and the timely application of biological agents or chemicals for population suppression where needed. Development of an effective integrated control strategy requires considerable knowledge of insect population behavior, tree damage, and regulatory factors operating in natural budworm systems.

Development of Microbial Insecticides

The use of biological agents (viruses, bacteria, fungi, or microsporidia) to control budworm is still in the research stage. The mode of action of *Bacillus thuringiensis* on eastern spruce budworm larvae has been under investigation in Canada for several years; initial field tests were made in 1959 (Angus et al., 1961; Smirnoff, 1963). Denton (1960) found in laboratory and field tests that *B. thuringiensis* when ingested in significant amounts, was toxic to larvae of the western spruce budworm.

Aerial applications of *B. thuringiensis* have demonstrated that it can cause considerable mortality of the budworm, but not at levels as high as those obtained with chemicals (Mott et al., 1961; Klein and Lewis, 1966).

Larval mortality caused by *B. thuringiensis* is due to an initiation of septicemia after ingestion of the bacterial spores. Septicemia does not begin until the bacteria have penetrated the gut wall and entered the hemolymph (Smirnoff, 1971). Addition of the enzyme chitinase (which hydrolyzes chitin) to *B. thuringiensis* preparations administered to budworm larvae increased the mortality rate attributable to the bacterium (Smirnoff, 1971). Recent aerial applications of a *B. thuringiensis* - chitinase formulation showed a larval mortality rate high enough to suggest the possibility of using such a formulation for budworm control (Smirnoff et al., 1973).

Development of Chemical Insecticides

Potential adverse environmental effects of DDT and related chemicals were recognized in the early 1960's. The last use of DDT for budworm control in the western United States was in 1964 on the Salmon National Forest in Idaho (Johnson and Denton, 1975). The Insecticide Evaluation Project was established in 1964 at the Pacific Southwest Forest and Range Experiment Station to find, test, and put into practical use insecticides effective against defoliators that were more environmentally acceptable than DDT.

Insecticides selected for screening agains the budworm were carbaryl, dimethoate, Zectran, pyrethrins, malathion, phosphamidon, and naled. These compounds were field tested for control effectiveness and possible side effects between 1963 and 1972 in several National Forests in Idaho and Montana (Johnson and Denton, 1975). Results of these field tests and larger scale pilot control projects led to the registration of malathion and Zectran. Results with other insecticides were inconclusive. The high cost and limited market for Zectran led to the end of its commercial production early in 1974.

Another carbamate insecticide, Matacil, exhibited systemic movement when injected into trees. These injections are claimed to be highly effective against the budworm (Chemagro Corp., 1970). It has also given effective control of budworm in small field tests of ULV aerial applications (Chemagro Corp., 1970). Matacil is still in the experimental stage.

APPENDIX C

BACKGROUND DOCUMENT FOR (R)DYLOX INSECTICIDE

by

W. Earlston Andrews[1]

Phyllis A. Dugar[2]

for

D. A. Pierce[3]

[1]Entomologist, Forest Pest Management Group, EPI Unit, NA-S&PF Portsmouth, New Hampshire.

2 & [3]Biological Laboratory Technician and Entomologist, respectively, Forest Pest Management Group, EPI Unit, SA-S&PF, Alexandria, Louisiana.

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PREFACE

Because of the interest in and the demand for more efficacious and environmentally compatible materials for the suppression of gypsy moth outbreaks, the following information has been compiled on trichlorfon (Dylox). The purpose of this compilation is to bring together in one relatively brief document, all of the pertinent information currently available on trichlorfon. At the present time, trichlorfon in the oil formulation is not registered for use in suppression of gypsy moth outbreaks. A number of tests are being conducted this year (1972) for the purpose of establishing the suitability of trichlorfon for use on the gypsy moth. Results from these tests will also provide additional information to support full registration. Dylox ULV spray (Ultra Low Volume) presently is registered for control of gypsy moth larvae. (USDA Reg. No. 31125-210).

BACKGROUND DESCRIPTION FOR PESTICIDE (R)DYLOX*

I. GENERAL INFORMATION

A. COMMON NAMES: trichlorfon (150), trichlorphon (Great Britain), dipterex (Turkey), Chlorofos (USSR).

OTHER NAMES: Anthon*, Bovinox*, Dipterex*, Equino-Aid*, Neguvon*, Tugon*, Trinox*, Bayer L13/59.

B. CHEMICAL NAME: Dimethyl (2,2,2, - trichloro-1-hydroxyethyl) phosphonate.

C. REGISTERED USES: Dylox is registered for use on a wide variety of field crops, vegetables, seed crops, and ornamentals. (Chemagro, Anon. 1971).

1. DYLOX 80% SP recommended for use on the following field crops:

(a) ALFALFA and CLOVER: To control alfalfa caterpillar, alfalfa webworm, western yellow-striped armyworm, beet armyworm, and variegated cutworm, lygus bugs, stink bugs, and tarnished plant bugs.

(b) BARLEY, FLAX, OATS, WHEAT: To control armyworms, beet webworm, variegated cutworm, beet armyworms, and diamondback moth.

(c) CORN: To control armyworms and cutworms.

(d) COTTON: To control cotton fleahopper, cotton leafworm, darkling ground beetle, western yellow-striped armyworm, beet armyworm, southern garden leafhopper, black fleahopper complex, cotton leaf perforator, leaf roller, lygus bugs, stink bugs, and salt-marsh caterpillar.

(e) SAFFLOWER: To control armyworms, lygus bugs, thrips, variegated cutworm.

*Indicates trade name.

(f) SUGAR BEETS: To control beet webworm, variegated cutworm, dipterous leaf miners, alfalfa webworm, beet armyworm, salt-marsh caterpillar.

(g) TOBACCO: To control budworm, hornworm, green june beetle larvae.

SEED FIELD CROPS

(a) ALFALFA AND CLOVER: To control armyworms, lygus bugs, stink bugs, and variegated cutworm.

(b) SOYBEANS: To control armyworms, dipterous leaf miners, lygus bugs, stink bugs, and variegated cutworm.

VEGETABLES

(a) BEANS (snap & dry): To control western bean cutworm, armyworms, dipterous leaf miners, lygus bugs, Mexican bean beetle, stink bugs, and variegated cutworm.

(b) LIMA BEANS: Same as above.

(c) COWPEAS (southern peas, blackeyed peas and crowder peas): Same as above.

(d) BRUSSEL SPROUTS, CABBAGE, CAULIFLOWER: To control western yellow-striped armyworm, imported cabbageworm, variegated cutworm, and diamondback moth.

(e) CARROTS: To control western yellow-striped armyworm, imported cabbageworm, variegated cutworm, diamondback moth, dipterous leaf miners, beet armyworm, lygus bugs, and salt-marsh caterpillar.

(f) COLLARDS AND LETTUCE: To control thrips, variegated cutworm, armyworm, beet webworm, diamondback moth, dipterous leaf miners and salt-marsh caterpillar.

(g) PEPPERS: To control dipterous leaf miners, pepper maggot, serpentine leaf miners.

(h) PUMPKIN: To control variegated cutworm, and squash bug.

(i) TABLE BEETS: To control variegated cutworm, alfalfa webworm, beet webworm, dipterous leaf miner, salt-marsh caterpillar, beet armyworm, and lygus bugs.

(j) TOMATOES: To control serpentine leaf miners, tomato hornworms, and dipterous leaf miners.

ORNAMENTALS

(a) FLOWERS, SHRUBS, AND TREES: To control nantucket pine tip moth, Zimmerman pine moth, armyworms, bagworms, climbing cutworms, dipterous leaf miners, lygus bugs, stink bugs, tarnished plant bug, tobacco budworm, and webworm.

(b) NARCISSUS: To control narcissus bulb fly.

(c) LAWNS AND TURF: To control sod webworms (lawn moths).

2. DYLOX ULTRA-LOW-VOLUME (ULV) SPRAY:

(a) FOREST AND SHADE TREES: Registered for use in gypsy moth control programs under supervision of government authorities. Registration application is pending in EPA for forest tent caterpillar.

3. DIPTEREX*: Registered for use as a fly bait in and around farm buildings, including dairy barns, milk processing rooms, and battery poultry establishments. It is also registered for control of cockroaches, crickets, silverfish, bed bugs, and fleas.

4. NEGUVON*: To control cattle grub, lice, and horn flies on beef cattle.

D. FORMULATIONS MANUFACTURED

Dylox is available in the following formulations:

80% Soluble Powder	5% Granular
95% Soluble Powder	5% Bait
80% SPA (Special)	ULV, 4# ai/gal
Liquid Solution, 4# ai/gal	Technical Grade

E. DILUTION OF FORMULATIONS FOR USE:

1. DYLOX ULV is used undiluted for gypsy moth control.

2. DYLOX 80% S.P.:

   (a) NANTUCKET PINE TIP MOTH AND ZIMMERMAN PINE MOTH: 20 ounces Dylox per 100 gallons of water.

   (b) OTHER INSECTS: Use 20-30 ounces Dylox per 100 gallons of water.

3. DYLOX LIQUID SOLUTION:

   (a) NANTUCKET PINE TIP MOTH AND ZIMMERMAN PINE MOTH: 2 pints L.S. concentrate per 100 gallons of water.

   (b) OTHER INSECTS: 2 to 3 pints of L.S. concentrate per 100 gallons of water.

4. DYLOX 80% SPA: Formulated specifically for use with summer crop spray oils of the 60 to 70 second viscosity type. The maximum amount of Dylox in oil recommended is 3 ounces actual Dylox/pint of oil (total volume). This pint mixture can be obtained by adding 3.75 ounces Dylox 80% SPA to 13.3 fluid ounces of oil.

F. RATE AND METHOD OF APPLICATION:

1. DYLOX ULV SPRAY:

   (a) FOREST AND SHADE TREES: Gypsy moth larvae; use 2 pints Dylox ULV spray per acre undiluted with suitable aircraft spraying equipment.

2. DYLOX 80% SOLUBLE POWDER: This formulation dissolves readily in water and is suitable for use in all power-operated ground sprayers and aircraft sprayers. Dosages below are expressed in terms of formulation.

FIELD CROPS

(a) ALFALFA and CLOVER:

(1) ALFALFA CATERPILLAR - Use 7½ to 10 ounces Dylox/acre using sufficient water for complete coverage but not less than 1 gallon/acre.

(2) ALFALFA WEBWORM - Use 5 to 20 ounces/acre.

(3) WESTERN YELLOW-STRIPED ARMYWORM - Use 10 ounces Dylox/acre.

(4) BEET ARMYWORM AND VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(5) LYGUS BUGS, STINK BUGS, AND TARNISHED PLANT BUG - Use 20 ounces Dylox/acre.

(b) BARLEY, FLAX, OATS, AND WHEAT:

(1) ARMYWORMS, BEET WEBWORM, VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(1) BEET ARMYWORM AND DIAMONDBACK MOTH - Use 20 ounces Dylox/acre.

(c) CORN:

(1) ARMYWORMS AND CUTWORMS - Use 10 to 20 ounces Dylox/acre.

(d) COTTON:

(1) COTTON FLEAHOPPER - Use 5 to 20 ounces Dylox/acre.

(2) COTTON LEAFWORM, DARKLING GROUND BEETLE, WESTERN YELLOW-STRIPED ARMYWORM - Use 10 to 20 ounces Dylox/acre.

(3) BEET ARMYWORM, SOUTHERN GARDEN LEAFHOPPER - Use 20 ounces Dylox/acre.

(4) BLACK FLEAHOPPER COMPLEX, COTTON LEAF PERFORATOR, LEAF ROLLER, LYGUS BUGS, AND STINK BUGS - Use 20 to 30 ounces Dylox/acre.

(5) SALT-MARSH CATERPILLAR - Use 30 ounces Dylox/acre.

(e) SAFFLOWER:

(1) ARMYWORMS, LYGUS BUGS, THRIPS, AND VARIEGATED CUTWORM - Use 20 to 30 ounces Dylox/acre.

(f) SUGAR BEETS:

(1) BEET WEBWORM, AND VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) DIPTEROUS LEAF MINERS - Use 20 ounces Dylox/acre.

(3) ALFALFA WEBWORM AND BEET ARMYWORM - Use 20 to 30 ounces Dylox/acre.

(4) SALT-MARSH CATERPILLAR - Use 30 ounces Dylox/acre.

(g) TOBACCO:

(1) BUDWORM AND HORNWORM - Use 20 ounces Dylox acre.

(2) GREEN JUNE BEETLE LARVAE - Use 10 ounces Dylox/acre.

SEED FIELD CROPS

(a) ALFALFA AND CLOVER:

(1) ARMYWORMS, LYGUS BUGS, STINK BUGS, AND VARIEGATED CUTWORM - Use 20 to 30 ounces Dylox/acre.

(b) SOYBEANS:

(1) ARMYWORMS, DIPTEROUS LEAF MINERS, LYGUS BUGS, STINK BUGS, AND VARIEGATED CUTWORM- Use 20 to 30 ounces Dylox/acre.

VEGETABLES

(a) BEANS (snap & dry):

(1) WESTERN BEAN CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) ARMYWORMS, DIPTEROUS LEAF MINERS, LYGUS BUGS, MEXICAN BEAN BEETLE, STINK BUGS, AND VARIEGATED CUTWORM - Use 20 to 30 ounces Dylox/acre.

(b) LIMA BEANS:

(1) WESTERN BEAN CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) ARMYWORMS, DIPTEROUS LEAF MINERS, LYGUS BUGS, MEXICAN BEAN BEETLE, STINK BUGS, AND VARIEGATED CUTWORM - Use 20 to 30 ounces Dylox/acre.

(c) COWPEAS (southern peas, black-eyed peas, and crowder peas):

(1) ARMYWORMS, DIPTEROUS LEAF MINERS, IMPORTED CABBAGE WORM, LYGUS BUGS, MEXICAN BEAN BEETLE, STINK BUGS, AND VARIEGATED CUTWORM- Use 20 to 30 ounces Dylox/acre.

(d) BRUSSEL SPROUTS, CABBAGE, AND CAULIFLOWER:

(1) WESTERN YELLOW-STRIPED ARMYWORM - Use 10 ounces Dylox/acre.

(2) IMPORTED CABBAGE WORM AND VARIEGATED CUT-WORM - Use 10 to 20 ounces Dylox/acre.

(3) DIAMONDBACK MOTH - Use 20 ounces Dylox/acre.

(e) CARROTS:

(1) WESTERN YELLOW-STRIPED ARMYWORM - Use 10 ounces Dylox/acre.

(2) IMPORTED CABBAGE WORM AND VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(3) DIAMONDBACK MOTH AND DIPTEROUS LEAF MINERS- Use 20 ounces Dylox/acre.

(4) BEET ARMYWORM AND LYGUS BUGS - Use 20 to 30 ounces Dylox/acre.

(5) SALT-MARSH CATERPILLAR - Use 30 ounces Dylox/acre.

(f) COLLARDS AND LETTUCE:

(1) THRIPS AND VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) ARMYWORMS, BEET WEBWORM, DIAMONDBACK MOTH, DIPTEROUS LEAF MINERS, AND SALT-MARSH CATERPILLAR - Use 20 ounces Dylox/acre.

(g) PEPPERS:

(1) DIPTEROUS LEAF MINERS, PEPPER MAGGOT, AND SERPENTINE LEAF MINERS - Use 20 ounces Dylox/acre.

(h) PUMPKIN:

(1) VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) SQUASH BUG - Use 20 ounces Dylox/acre.

(i) TABLE BEETS:

(1) VARIEGATED CUTWORM - Use 10 to 20 ounces Dylox/acre.

(2) ALFALFA WEBWORM, BEET WEBWORM, DIPTEROUS LEAF MINERS, AND SALT-MARSH CATERPILLAR - Use 20 ounces Dylox/acre.

(3) BEET ARMYWORM AND LYGUS BUGS - Use 20 to 30 ounces Dylox/acre.

(j) TOMATOES:

(1) SERPENTINE LEAF MINERS, TOMATO HORNWORMS, AND DIPTEROUS LEAF MINERS - Use 20 ounces Dylox/acre.

ORNAMENTALS

(a) FLOWERS, SHRUBS, AND TREES:

(1) NANTUCKET PINE TIP MOTH AND ZIMMERMAN PINE MOTH - Use 20 ounces Dylox/100 gallons of water. Spray plants until thoroughly wet.

(2) ARMYWORMS, BAGWORMS, CLIMBING CUTWORMS, DIPTEROUS LEAF MINERS, LYGUS BUGS, STINK BUGS, TARNISHED PLANT BUG, TOBACCO BUDWORM, AND WEBWORMS - Use 20 to 30 ounces Dylox/acre/100 gallons of water. Spray plants until thoroughly wet.

(b) NARCISSUS:

(1) NARCISSUS BULB FLY - Use 20 ounces Dylox/100 gallons of water. Apply as a drench per 1,000 feet of row.

(c) LAWNS AND TURF:

(1) SOD WEBWORMS (lawn moths) - Use 2 1/2 to 3 3/4 ounces Dylox/15 to 30 gallons of water. Apply uniformly to 1,000 sq. ft. of lawn or turf by means of a watering can, compressed air sprayer, or any commercial power sprayer.

3. NEGUVON:

(a) BEEF CATTLE AND NONLACTATING DAIRY CATTLE: Use solution at the rate of ½ fluid ounce per 100 lbs. of body weight. Apply by a pour-on technique.

4. ANTHON: This formulation is used only on horses as a wormer. The dosage rate is one packet (5 grams) per 250 lbs. of body weight. It is to be administered as a dry dosage or dissolved in water and mixed with the amount of feed to be consumed in one feeding.

G. TOLERANCES IN FOOD OR FEED:

Tolerance levels ranging from 0.1 PPM in certain vegetable crops up to 45 PPM in the hay of certain field crops, 0.1 PPM in meat, fat and meat by-products and 0.01 PPM in milk of cattle have been established for residues of Dylox.

H. SAFETY LIMITATIONS:

1. DYLOX ULV SPRAY: Do not apply to crops used for feed or forage.

2. DYLOX 80% SOLUBLE POWDER: Do not use on crops used for feed or forage. Do not treat food crops grown in greenhouse.

3. NEGUVON: Do not apply in conjunction with oral drenches, other internal medications, or with other organic phosphates or materials having cholinesterase inhibiting activity.

   Do not treat lactating dairy cattle, animals less than 3 months old, sick, convalescent, or stress livestock.

4. ANTHON: Do not treat horses to be used for food. Do not treat debilitated horses, colts under 4 months of age, mares in the last month of pregnancy, or animals other than horses.

I. MANUFACTURER OR PRODUCER:

Chemagro Division of Baychem Corporation, Kansas City, Missouri.

II. TOXICITY DATA ON FORMULATION TO BE USED:

A. SAFETY DATA: Dylox, like other organophosphates, manifests its toxicity by depression of cholinesterase. Under experimental conditions, rather rapid recovery from toxic symptoms has been noted, indicating rapid reversal of cholinesterase depression, (Chemagro Anon. Jan. 1971).

1. ACUTE MAMMALIAN STUDIES:

(a) ORAL: Levine, et al. (1958) determined the approximate LD_{50} of Dipterex on mice to be 600-800 mg/kg. Riek and Keith (1958) in studies of anthelmintics for cattle administered Bayer L13/59 to 200 calves at rates up to 5 g/100 lbs. body weight without mortality. In a related study, laboratory calves were treated with dose rates up to 12.5 g/100 lbs. without mortality, except in one instance in which death occurred at a dose rate of 10 g/100 lbs. of body weight.

Chemagro, Anon. (Jan. 1971) gives the oral LD_{50} expressed in mg/kg of body weight for the various formulations to rats and mice: Technical (rats--male and female) 450-500, Technical (mice--male and female) 950, ULV-4 lb/gal (rats and mice--female) 35-45, liquid solution--4 lb/gal (rats--female) 950, 80% Soluble Powder (rats--female) 450-500, and 80% SPA (rats--female) 2500.

(b) DERMAL: Chemagro, Anon. (Jan 1971) gives the dermal LD_{50} expressed in mg/kg for three formulations of Dylox: Technical (rats--male and female)>2000, Liquid Solution--4 lb/gal (rats--female)>2000, and 80% soluble powder (rats--female)>2000.

Williams, et al. (1959) reports that DuBois and Cotter (1955) determined cholinesterase on brain, serum, and submaxillary glands of rats following single acute interperitoneal administration of 25, 50, and 125 mg/kg. There was transient depression at all levels and in all three tissues with relatively complete return to pretreatment levels within 5 hours.

(c) INHALATION: Chemagro, Anon. (Jan 1971) gives the inhalation LC_{50} expressed in ug/L for various Dylox formulations: Technical (rats--male and female)>1000, technical (mice--male and female)>10,000, ULV--4 lb/gal (rats-female) 3,600 and (mice-female) 2,400 and 80% soluble powder (rats--female)>20,000.

(d) EYE AND SKIN IRRITATION: The warning precautions on labels for Dylox formulations state "Do not get in eyes, or skin, or on clothing". No other information available.

2. SUBACUTE MAMMALIAN STUDIES: DuBois (Chemagro Report 3170, January 1954), administered daily intraperitoneal injections of aqueous solutions of Dylox to adult female Sprague - Dawley rats (three groups of five rats each). All of the rats were able to tolerate 50 mg/kg of Dylox daily for 60 days without mortality. After 100 mg/kg was administered, but all of a group of five animals succumbed to daily injections of 150 mg/kg.

Williams, et al. (1959) states that Deichmann and Lampe (1955) found, when feeding two dogs 42 mg/kg of Dipterex (i.e. 10% of a lethal oral dose) 6 days a week for three months, that the average plasma cholinesterase had fallen to 59.5% of the control level, while no overt signs of intoxication were noted.

Two male and female dogs, of mixed breed weighing 6-10 kg were placed on each of three levels of Dipterex, 50, 200, and 500 ppm of total diet. Four control dogs were studied simultaneously. There was significant depression in both plasma and red-cell cholinesterase within 2 weeks at 500 ppm, and borderline depression at 200 ppm, while no depression was noted at 50 ppm. (Williams, et al. 1959).

3. OTHER STUDIES:

(a) NEUROTOXICITY: In a study (Levine et al. 1958) of its anthelmintic value, Dipterex was administered in a gelatin capsule with a balling gun to sheep. In doses of 100-200 mg/kg no neuromotor symptoms were produced. A 400 mg/kg dose produced typical neuromotor symptoms followed by recovery.

At dosages of 5000 ppm, 2000 ppm, 1000 ppm, and 500 ppm in connection with a 30-day feeding period, Dipterex caused no histological changes in the spinal cord and sciatic nerves of chickens. No histological changes that could be attributed to Dipterex were observed in those animals killed immediately after the feeding period (Hobik, H.P. 1967-- Chemagro Report 21363). Likewise, in acute

and subacute tests, Kimmerle (1966), was unable to detect neurotoxic damage in chickens due to application of Dipterex active ingredient.

B. TERATOGENICITY: A study was conducted on the teratogenic effects of Dipterex on rats (Lorke, D., 1971). Twenty pregnant rats per group were used in this study. From the 6th to the 15th day of pregnancy, Dipterex was administered by means of a stomach tube in the following manner: 0 mg/kg of body weight, 10 mg/kg of body weight, 30 mg/kg of body weight, and 100 mg/kg of body weight.

Dipterex was found to be harmless to the mother animals up to the dosage of 30 mg/kg of body weight. In the higher dosage, diarrhea occurred in 4 out of 20 rats; however, no other deviations from normal behavior were detected.

The fetuses were removed from the mother rats and examined for external malformations and weight. There were no significant differences noted between treated animals and those of the control group. It is therefore concluded that Dipterex administered in proportions up to 30 mg/kg of body weight to rats had no teratogenic effects.

C. REPRODUCTIVE EFFECTS: Pregnant ewes were treated with an oral dose of Neguvon (Dipterex) at the rate of 2.5 g/100 lbs. of live weight at about one month and again at two weeks before lambing commenced. No abortions were observed and apparently the ewes lambed normally. (Southcott, 1961).

A similar experiment was repeated on rats.

D. SYNERGISM: No information available.

E. CARCINOGENICITY: A 4 year study by the Huntington Research Centre, Huntington, England, utilizing dogs as subject animals concluded that there were no changes in morphology which were considered to have been induced by the feeding of technical grade Dylox.

F. METABOLISM: A study was made by Robbin, et al. on the effects Dylox has on the metabolism of a lactating Hereford cow. P-32 labeled Bayer L13/59 was administered orally at the rate of 25 mg/kg in 100 ml of distilled water. Thirty minutes after administration, the concentration of radioactivity in the blood was 8.8 ug-equivalents/ml. The peak of radioactivity was attained between the first and third hours after treatment with a recorded maximum of 15.1 ug-equivalents/ml at 2 hours.

Relatively low levels of radioactivity were found in milk. The maximum concentrations were detected in the 6-48 hour samples, with a peak of 2.3 ug-equivalents/ml. At 13 hours after administration, there was a constant decrease to 0.77 ug-equivalent/ml. At the end of 144 hours, less than 0.2% of the administered dose, as determined radiometrically, had been secreted in the milk.

Trace amounts of radioactivity were first detected in the feces about three hours after treatment. Radioactivity gradually increased until a peak was reached in 18 hours.

Bayer L13/59 and/or its metabolites are very rapidly excreted via the urine. One and ¼ hours after the first treatment, the urine contained 0.54 mg-equivalent/ml. The peak was reached at 2½ hours after administration with a concentration of 1.4 mg-equivalent/ml; concentration at 5½ hours after administration was 1.38 mg-equivalent/ml.

Studies following topical application on the leaf, as well as via root, of the translocation and metabolism of 32PC, labeled Dipterex in cotton plants were made by Mostafa et al. (1969). The insecticide did not penetrate into the leaf cell, when applied topically, but was readily taken up by the root when immersed in a radioactive solution of the insecticide. Rate of respiration was found to increase significantly in plants treated with sublethal concentration of Dipterex.

Based on previous studies, Hassan, et al., (1966), concluded that the detoxification of the insecticide "In Vivo" proceeded as follows:

(1) In the rat; exclusive hydrolysis of the phosphonate bond of both Dipterex and its demethylated metabolite.

(2) In prodenia larvae; 70% hydrolysis of O-methylester linkage and 30% splitting of C-P bond.

(3) In cotton plant - hydrolysis of the phosphonate bond followed by demethylation of the methylated phosphates.

(4) In micro-organisms: exclusive hydrolysis of O-methylester bond(s).

G. AVIAN AND FISH TOXICITY:

BIRDS

In a test by DeWitt, et al., (1960), trichlorfon was reported more toxic to quail and pheasants than carbaryl. The LD_{50} for pheasants, for instance, with trichlorfon was 2,500 mg/kg, while with carbaryl, it was over 40,000. The maximum trichlorfon concentration in the diet permitting normal survival was 100 ppm for quail and 500 ppm for pheasants. In a Bobwhite quail feeding test employing 134 ppm (approximating 2 pounds of trichlorfon per acre), brain acetylcholinesterase inhbition was 38 percent after 24 hours exposure, 48 percent after 48 hours exposure, and 63 percent after 96 hours. Investigations during 1961-62 (U.S.D.I. Stewart L. Udall) revealed quantities of Dipterex causing 50 percent mortality for some species of birds. Species as follows: (Test period less than 10 days).

	YOUNG	
Bird	PPM. in Diet	MG/KG Eaten
Bobwhite	750	425
Ring-Necked Pheasant	5,000	2,500

Circular 143 of Bureau of Sport Fisheries and Wildlife reports the approximate lethal dose or levels of Dipterex in diet that reduced reproduction by 25% or more for the following birds are:

Bird	Approximate Lethal Dose (mg/kg)		Level in Diet Reducing Reproduction 25% or more (p.p.m.)
Bobwhite	Young	Adult	
	600	4,000	25
Pheasant	Young	Adult	
	2,600	800	25

Pearce (1970) in New Brunswick reports that two aerial applications of six ounces active ingredient per acre did not present a significant hazard to the American redstart, Tennessee warbler, and Blackburnian warbler. Chemagro Corporation (Anon. 1971b) reported that in a study where 1.25 pounds active ingredient per acre was applied, doves, quail, blackbirds, and meadowlarks were observed before, during and after spraying and appeared unaffected by the application. The LD_{50} range of 28-50 mg/kg for redwinged blackbirds and starlings is lower than for quail and pheasants.

FISH

In a test by Pickering, et al., (1962), trichlorfon was found to be the safest organic phosphorous formulation administered to fathead minnows and bluegills. In a test by Hoffman (1957) trichlorfon did not affect fingerling and legal size rainbow trout exposed for 24 and 72 hours to 1.0 and 10.0 ppm. In all cases, the fish were held for at least 48 hours after the toxicant was discontinued for observation of possible delayed effects. Matton and Laham studied the effect of Dylox on immature rainbow trout. They treated 1-inch rainbow trout for 16 hours with from 10-100 ppm Dylox or for 40 hours with 5 ppm. This treatment produced a marked acetylcholinesterase inhibition that was reflected by their abnormal behavior pattern. Histological examination revealed pathological changes which could not be explained on basis of acetylcholinesterase inhibitions.

Lewallen and Wilder (1962) fed rainbow trout 1.0 and 10.0 ppm and found no harmful effects or mortality at 24, 48, and 72 hours. Chemagro (Anon. 1971b), however, reported that fingerling rainbow trout exposed to concentrations of 10.0 ppm died within 24 hours. Hornbeck, et al. (1965), used applications of

0.25 ppm Dylox to test possible chemical control for tadpole shrimp. Results indicated that large mouth bass and channel catfish, bass and bluegill were unaffected by the treatment. Weiss (1961) states that trichlorfon at a concentration of 1.0 ppm caused 50 percent mortality of golden shiners after six hours. Trichlorfon has a low enough toxicity to fish and a high enough toxicity to fish parasites that it has been explored as a possible treatment for such parasites.

H. PHYSICAL - CHEMICAL PROPERTIES OF TECHNICAL:

1. BOILING POINT: 100°C 0.1 mm. Hg.

2. MELTING POINT: 81 to 82°C. Technical does not have flash point.

3. PHYSICAL STATE: White crystalline solid.

4. DENSITY: Molecular Weight - 257.6

   Specific gravity - 1.73 $\frac{20^{\circ}}{40}$C

5. VAPOR PRESSURE: Volatility - 0.1 mg/cu meter 20°C
   2.0 mg/cu meter 40°C

6. SOLUBILITY: Soluble in Water - CA. 12% 26°C
   CA. 9% 20°C
   CA. 7% 15°C
   CA. 5% 10°C
   CA. 3% 5°C

   Soluble in alcohols, methylene chloride and keytones. Slightly soluble in aromatic solvents.

7. STABILITY: Subject to hydrolysis and dehydrohalogenation. Decomposition proceeds more rapidly with heating and above PH6.

8. COMPATIBILITY WITH OTHER CHEMICALS: Known to be compatible with chlorinated and phosphate insecticides. Incompatible with alkaline materials such as lime and lime sulfur.

9. POTENTIATION: Of the cholinesterase inhibiting pesticides for which tolerances have been established, Dylox causes acute potentiation with only Guthion and Malathion. However, feeding dogs and rats on

diets containing "no effect" levels of Dylox and each of the other compounds did not result in potentiation. (Chemagro, Anon. 1971).

that at an exposure of 1 ppm, trichlorfon had no effect on the productivity of phytoplankton. Chemagro Corporation (Anon., 1968) reported that trichlorfon has no effect on phytoplankton exposed to 10 ppm. Dylox was fed to goldfish at successive levels of 100, 1,000, and 2,500 ppm. No effects were noted on copepods or rotifers in the pond. Cladocorans were apparently eliminated. As a pond application at 1.00 ppm in tests for control of anchor worm parasites of fish, Dylox caused a serious reduction of the zooplankton population. Survival of fish fry in Dlyox-treated ponds was highest in the test. Daphnia cultures were exposed to 0.10, 0.25, 0.50, and 1.0 ppm of Dylox in laboratory tests conducted in 100 mm petri dishes. All concentrations immobilized Daphnia within two hours. Dylox, like all other insecticides, does exert a drastic effect on zooplankton, especially crustaceans such as pink shrimp, fairy shrimp, tadpole shrimp, copepods, and Daphnia. However, unlike other insecticides, the effects of Dylox on zooplankton are short-lived and populations recover rapidly. (Chemagro, Anon., 1968).

B. RESIDUES IN OR ON FOOD OR FEED OR ENTERING INTO FOOD CHAIN VIA AIR, WATER, SOIL, PLANTS OR ANIMALS: Shorey, et al., evaluated the residues remaining on numerous vegetable crops after Dylox application. Pepper, pea pods, and the leafy portion of beets, turnips and lettuce contained residues greater than 0.15 ppm, 7 days after application. With the exception of pea pod, no residues in excess of 0.15 ppm were detected on any of the crops 14 days after application.

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[illegible] Species [illegible]

[illegible] Facts of [illegible] University [illegible] York, Progress [illegible]

[illegible] Phosphate [illegible]

APPENDIX D

THE IMPACT OF ORTHENE ON THE ENVIRONMENT

This report will show that ORTHENE (acephate), a new organophosphorus insecticide, has an extremely soft impact on the environment for the following reasons:

1. It is of low toxicity to mammals, birds, fish and soil microorganisms.

2. It is rapidly degraded in the biosphere to innocuous products.

3. It is not bioconcentrated.

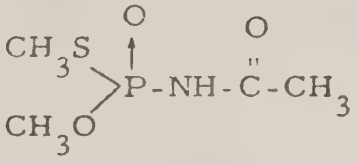
January 1973

THE IMPACT OF ORTHENE ON THE ENVIRONMENT

GENERAL DATA PERTINENT TO THE ENVIRONMENTAL IMPACT OF ORTHENE (Section 1)

Structure and Nomenclature

ORTHENE is an economic organophosphorus insecticide of the following chemical name and structure:



O,S-Dimethyl acetylphosphoramidothioate

The common name for ORTHENE is acephate. ORTHENE is a registered trademark of Chevron Chemical Company. In many of the accompanying documents, the words "ORTHENE", "ORTHENE Insecticide" and "acephate" are used interchangeably. ORTHENE has also been known as ORTHO 12,420, RE 12,420 and ENT 27822 and is also referred to by these names in some of the accompanying documents.

Physical Properties

ORTHENE is a white crystalline solid, mp 92-93°C (pure) and 82-89°C (technical). Its vapor pressure is very low (2×10^{-6} mm Hg at 25°C) (Ref. 1.1).

ORTHENE has very high solubility in water (65%), high solubility in polar organic solvents (e.g., methanol 25%; methylene chloride 30%), and low solubility in hydrocarbon solvents (e.g., benzene 1%; hexane 0.01%) (Ref. 1.2).

Concentration in Air

From the vapor pressure it can be calculated that at ambient temperatures the concentration of ORTHENE in saturated air would be 0.02 mg/m^3 or 0.002 ppm (Ref. 1.1).

This very low vapor concentration is further verified by a study of the volatility of ORTHENE from leaves (Ref. 1.3). ^{14}C-ORTHENE was topically applied as an aqueous solution to a bean plant, which was then housed in an

enclosed system. Air was passed over the plant and any volatile radioactivity was trapped and counted. In 21 hours no radioactivity (less than 0.002% of the amount applied) was detected in the trap. Thus, ORTHENE is not volatile from leaf surfaces and does not codistill with the spray or transpiration water.

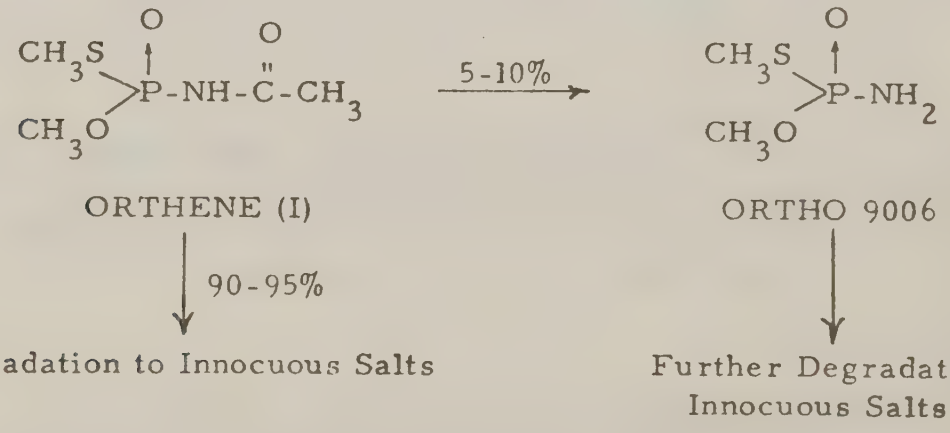
ORTHENE, therefore, should not contaminate the air nor pose any vapor hazard to animals, including man.

Effect of Light

ORTHENE dissolved in water or deposited on glass or paper is stable to sunlight (Ref. 1.4). ORTHENE samples exposed to simulated sunlight (GE type RS sunlamp, 275 W) exhibited the same degree of degradation as samples left in the dark. Thus, light is not a major factor in the consideration of the effect of ORTHENE on the environment.

PLANT METABOLISM (Section 2)

Studies on both field sprayed crops and using ^{14}C-labeled ORTHENE (I) in the greenhouse have shown that ORTHENE is readily degraded by plants (Ref. 2.1, 2.2, 2.3). The half-lives observed are generally 5-10 days. From these studies the following decomposition sequence can be written:



ORTHO 9006 (II) is O,S-dimethyl phosphoramidothioate. It is registered as an economic insecticide under the name of MONITOR ®. Detailed reports on chemical, metabolic, residual and toxicological properties are to be found in Residue Tolerance Petition No. 0F0956. ORTHO 9006 is degraded at about the same rate as is ORTHENE (Ref. 2.4).

From kinetic studies, it was found that only about 5-10% of the ORTHENE degrades via ORTHO 9006; the remainder degrades directly to innocuous salts. [illegible]abolite of toxicological significance other than ORTHO 9006 has been

ORTHENE (and of ORTHO 9006) are those in which the P-N, P-O, and/or P-S bonds are broken, yielding P-OH acids. According to D. F. Heath (Organophosphorus Poisons, Pergamon Press, 1961, p. 5), conversion of any of these bonds to the P-OH group is sufficient to detoxify the compound completely.

ORTHENE and ORTHO 9006 are adsorbed onto and/or absorbed into leaf surfaces. Water washing of field treated broccoli, lettuce and cotton leaves removed no more than 5% of the ORTHENE or ORTHO 9006 residues (Ref. 2.6, 2.7, 2.8). The data are summarized in Table I.

TABLE I

The Effect of Washing on Weathered Residues of ORTHENE and ORTHO 9006
(All Samples Received 2 Lb ORTHENE/Acre)

T-No. /Crop	Days*	Chemical	Washed Crop (ppm)	Washings (ppm)	Average % Removed
	3	ORTHENE	4.32	0.14	3
			3.94	0.15	
		ORTHO 9006	0.64	0.02	4
			0.45	0.02	
T-2051	7	ORTHENE	3.29	0.14	4
Lettuce			2.69	0.13	
		ORTHO 9006	0.79	0.03	4
			0.57	0.03	
	14	ORTHENE	1.96	0.11	4
			1.90	0.07	
		ORTHO 9006	0.45	0.03	5
			0.47	0.02	
	3	ORTHENE	12.4	1.64	12
			12.4	1.68	
		ORTHO 9006	1.34	0.09	6
			1.44	0.09	
T-2052	7	ORTHENE	8.95	0.48	5
Broccoli			8.05	0.55	
		ORTHO 9006	1.14	0.04	3
			1.08	0.03	
	14	ORTHENE	4.11	0.13	4
			4.02	0.15	
		ORTHO 9006	0.83	0.02	2
			0.75	0.01	

*Last spray to harvest

TABLE I (Continued)

T-No. /Crop	Days*	Chemical	Washed Crop (ppm)	Washings (ppm)	Average % Removed
	0	ORTHENE	371	14	3
			371	12	
		ORTHO 9006	31	0.38	1
			30	0.33	
	7	ORTHENE	179	6.7	3
T-2071			185	4.0	
Cotton		ORTHO 9006	22	0.26	1
Leaves			24	0.23	
	14	ORTHENE	93	2.3	2
			165	2.6	
		ORTHO 9006	17	0.16	1
			24	0.24	
	21	ORTHENE	58	1.4	4
			70	4.0	
		ORTHO 9006	13	0.24	3
			15	0.53	

*Last spray to harvest

Translocation studies using S-methyl-^{14}C-ORTHENE and ORTHO 9006 have shown that there is only slight movement of the chemical from a treated leaf to other parts of the plant, including into the roots and tubers (Ref. 2.9, 2.10, 2.11). Field studies with potatoes and sugar beets confirm this. At several weekly applications at 1 lb/acre, there is a maximum of only about 0.2 ppm ORTHENE and 0.04 ppm ORTHO 9006 in the potato tubers and slightly less in the sugar beet roots.

Both chemicals are readily picked up by plants from treated soil (Ref. 2.10). From soil treated at 10 ppm, radish tops contained 46 ppm ORTHENE and 12 ppm ORTHO 9006. The roots contained only 1.9 ppm ORTHENE and 0.6 ppm ORTHO 9006. These results are in agreement with previous studies which indicated that ORTHENE and ORTHO 9006 are moved to the leaves in the transpiration stream and there accumulate as water is transpired.

FATE AND METABOLISM IN SOIL (Section 3)

Rate of Degradation

ORTHENE is rapidly degraded in soil (Ref. 3.1, 3.2). Nine soils have been studied in the laboratory. Table II summarizes their classifications, origins, and half-lives of ORTHENE observed at 1 and 10 ppm fortification levels under aerobic conditions. The soils were wet to about field capacity.

TABLE II

The Degradation of ORTHENE in Soil

Soil Origin	Soil Type	Half-Life (Days)	
		1 ppm Fort.	10 ppm Fort.
Clarkesburg, Ca.	Clay	1-1/2	1-1/2
Fresno, Ca.	Loam	1-1/2	3
Kettleman City, California	Clay (high pH)	1/2	1/2
Ocoee, Florida	Loamy Sand	1	1
Mt. Holly, N. J.	Sandy Clay Loam	1/2	1
Norwalk, Iowa	Silty Clay Loam	--	2
Greenville Mississippi	Clay (low pH)	--	1-1/2
Ocoee, Florida	Muck	6	13
Riverside, Ca.	Loamy Sand	--	4*

*20 ppm fortification

In 8 out of the 9 soils, ORTHENE is degraded with half-lives ranging from 1/2 to 4 days. In the other soil, a very high organic muck soil, the half-life is 6 to 13 days.

ORTHO 9006 is also degraded in soil and has a half-life range of 2 to 6 days (Ref. 3.3).

Both chemicals degrade more rapidly in wet soil than in dry soil (Ref. 3.4). The data are summarized in Table III.

TABLE III

Comparison of ORTHENE and ORTHO 9006 Stability in Wet Versus Dry Soil

Soil	Soil Moisture	Half-Life (Days)	
		ORTHENE	ORTHO 9006
Fresno Loam	12.9%	3	1/2
	1.6%	11	1-1/2
Mt. Holly Sandy Clay Loam	20.3%	1	1/4
	6.5%	3	2/3

In addition to laboratory tests, soil samples from field sprayed crops have been analyzed for ORTHENE and ORTHO 9006. Although the rates of degradation in the field are slower than in the lab, in general these field data confirm the rapid degradation of both ORTHENE and ORTHO 9006 in soil. The results are summarized in Tables IV (ORTHENE) and V (ORTHO 9006). [See the ORTHENE Residue Tolerance Petition (Ref. 2.3) for experimental details of these tests.]

It is concluded that there will be no buildup of ORTHENE or ORTHO 9006 in soil from one season to the next.

Uptake from Soil by Crops

As discussed above under PLANT METABOLISM, ORTHENE and ORTHO 9006 can be picked up by plant roots and translocated to the aerial portions of the plant. The soil half-lives suggest that crops grown following a treated crop or the following season will not be contaminated, however, with residues of either chemical.

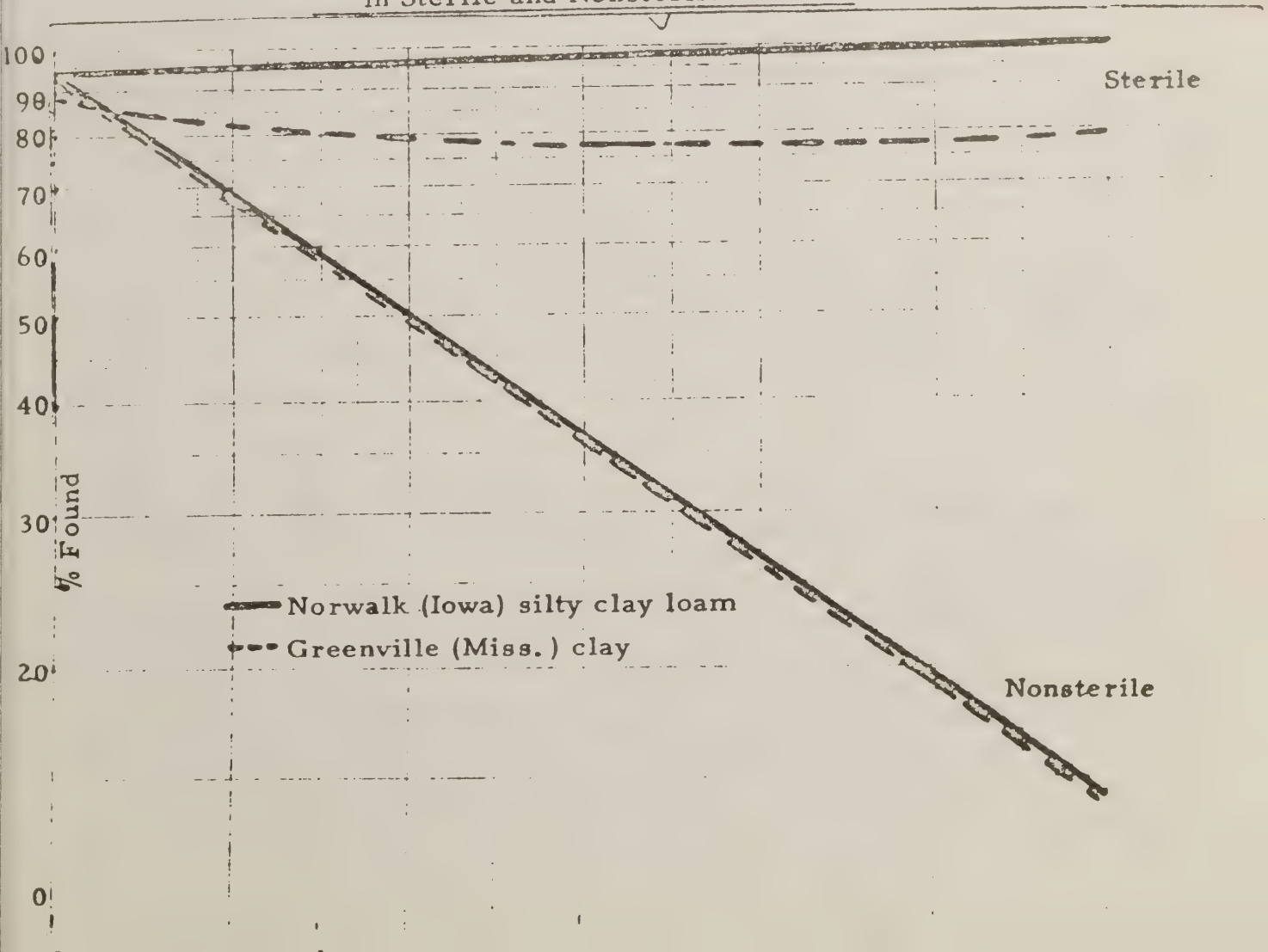
A study using ^{14}C-ORTHENE and following the EPA protocol was conducted to determine the amount of ORTHENE and/or its metabolites picked up from aged treated soil (Ref. 3.5). Soil in pots was treated with 2 ppm ^{14}C-ORTHENE and allowed to age in the greenhouse for 90 days. The soil was kept moist during this time. Carrots were then seeded into the soil and allowed to grow. Both immature and mature carrots were harvested and analyzed for radioactivity. The maximum amount of radioactivity found in either the roots or tops was equivalent to 0.005 ppm ORTHENE, an insignificant amount.

To study this in the field, crops have been grown in soil containing trash from a previous crop which had been sprayed with ORTHENE. The results are summarized in Table VI. [See Section D of the ORTHENE Residue Tolerance Petition (Ref. 2.3) for the details.] Except for one test, no residues were detected in the follow-up crops. In that one test (T-2307) up to 0.07 ppm ORTHENE, but no ORTHO 9006, was detected in mustard greens. (Note that in this test the crops were planted 11 days after the last treatment.) No residues were detected in 2 other crops (tomatoes and goosegrass) from the same plots. Thus, there is no or insignificant carry over of ORTHENE or ORTHO 9006 from a treated crop to a follow-up crop, even when it is planted within 2 weeks of the last ORTHENE treatment.

Soil Microorganisms

The decomposition of both ORTHENE and ORTHO 9006 in soil is biological, i.e., due to soil microorganisms (Ref. 3.1, 3.3). Figure 1 shows the relative rates of decomposition of ORTHENE in two different soil types under sterile and nonsterile (natural) conditions.

FIGURE 1
Rates of Decomposition of ORTHENE
in Sterile and Nonsterile Soils



The effect of ORTHENE and ORTHO 9006 on soil organisms is discussed in Section 6.

Metabolic Pathway

Soil treated with S-methyl-^{14}C-ORTHENE releases $^{14}CO_2$ as the ORTHENE concentration decreases (Ref. 3.1). As in plant metabolism, the only other toxicologically significant metabolite observed is ORTHO 9006. This chemical does not concentrate in soil, but is itself rapidly degraded (Ref. 3.1 and cf. the MONITOR tolerance petition). The proportion of ORTHENE that degrades through ORTHO 9006 is a little higher in soil than in plants. Table VII summarizes the data obtained in the soil metabolism study.

TABLE VII

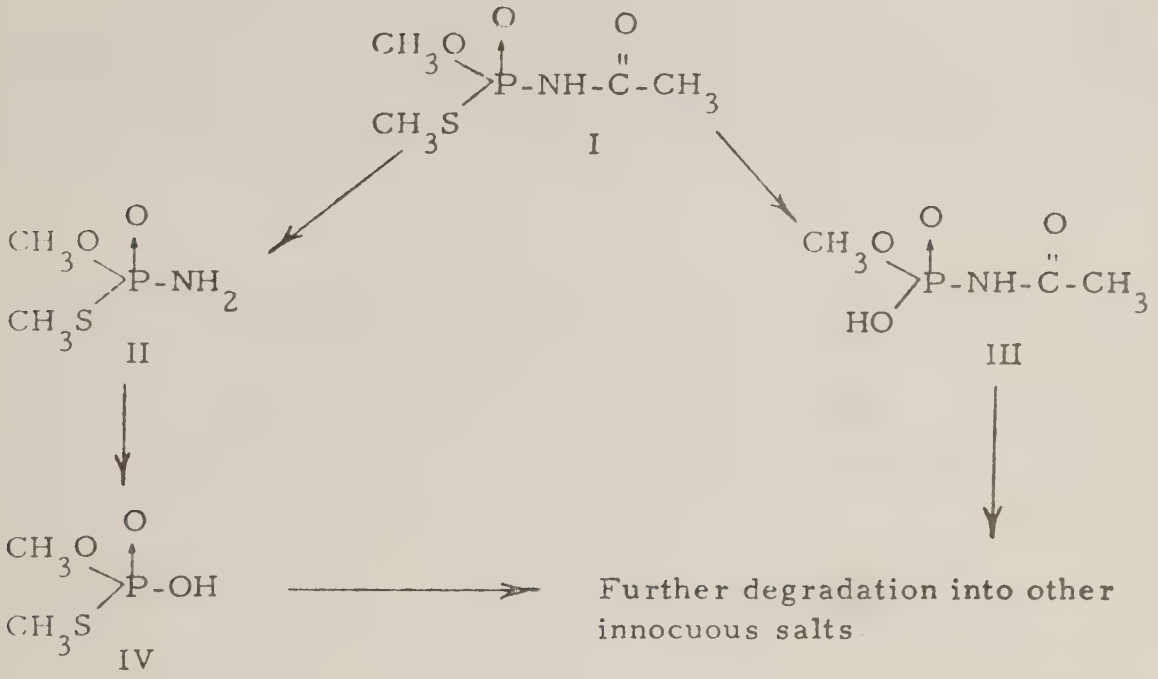
Soil Metabolism of S-Methyl-^{14}C-ORTHENE

Soil	Interval Treatment to Analysis (Days)	Percent of Initial ^{14}C Soil Treatment				
		$^{14}CO_2$	Total ^{14}C in Soil	^{14}C Extr. with MeOH	Extract Chromatography	
					ORTHENE	ORTHO 9006
Fresno (Calif.) Loam	1	9	90	84	63	21
	2	20	74	66	43	23
	6	54	47	26	NA*	NA*
Mt. Holly (N. J.) Sandy Clay Loam	1	42	54	43	36	7
	2	57	33	21	18	3
	6	76	17	2	NA*	NA*
Norwalk (Iowa) Silty Clay Loam	1	67	35	23	19	4
	2	81	22	6	5	1
	6	86	17	NA*	NA*	NA*

*NA - Not Analyzed

Methyl acetylphosphoramidate (III) has also been identified as a soil metabolite. Initially most of the dose can be extracted with methanol and is either ORTHENE or to a lesser extent ORTHO 9006 and III. As time from treatment increases, less radioactivity is extracted. The radioactivity remaining in the soil is most probably ^{14}C incorporated into the organic part of the soil or the microorganisms.

The degradation route of ORTHENE in soil can be shown by the following diagram



Compound IV (O,S-dimethyl phosphorothioate) has been identified as a plant and soil metabolite of ORTHO 9006, but not directly from ORTHENE (Ref. 2.4, 3.3).

The metabolic route of ORTHENE in soil under anaerobic conditions is the same as under aerobic conditions (Ref. 3.6). Only the rate is different, being slightly slower under anaerobic conditions. Less $^{14}CO_2$ is released, and from the extraction data, it appears that more of the ^{14}C label is incorporated into natural constituents of the microorganisms than under aerobic conditions.

Movement in Soils

Laboratory studies using both column leaching and soil thin-layer techniques have shown that ORTHENE and ORTHO 9006 are readily moved by water in soil with little retention by the soil particles (Ref. 3.7, 3.8). There is essentially no difference in the rate of leaching of ORTHENE in soil wet to field capacity or in air-dried soil (Ref. 3.4).

The leaching of "weathered" (aged) soil residues has also been studied following EPA protocols (Ref. 3.9). ^{14}C-ORTHENE was applied to soil at 2 ppm and the soil incubated moist for 20 days. About 15% of the applied ^{14}C remained in the soil after the incubation period. The soil was then placed on top of columns of the same soil (but untreated) and leached with 1/2-inch of

water daily for 46 days. Only 0.3% of the applied ^{14}C was recovered in the leachates. The maximum concentration of ^{14}C in the leachates was 0.001 ppm calculated as ORTHENE. Following leaching, the residual radioactivity was found to be almost entirely in the top 3 inches. Furthermore, only about 0.1% of the initial dose of ^{14}C was extractable from this leached soil column. Thus after aging, the soil ^{14}C residues are neither leachable nor are they ORTHENE or ORTHO 9006, but represent incorporation of the ^{14}C into natural constituents.

In another aging test, soil samples were sprayed with ORTHENE at 9 lb/Acre and allowed to age undisturbed except for watering for 21 weeks in the greenhouse (Ref. 3.10). At the end of this period, the soil contained 0.05 ppm ORTHENE or less and no ORTHO 9006. This is about 0.5% of the applied dose. This remaining ORTHENE was entirely leachable with the equivalent of 10 inches of rain. No bound ORTHENE or ORTHO 9006 was present in the soil.

FATE IN WATER (Section 4)

Hydrolysis

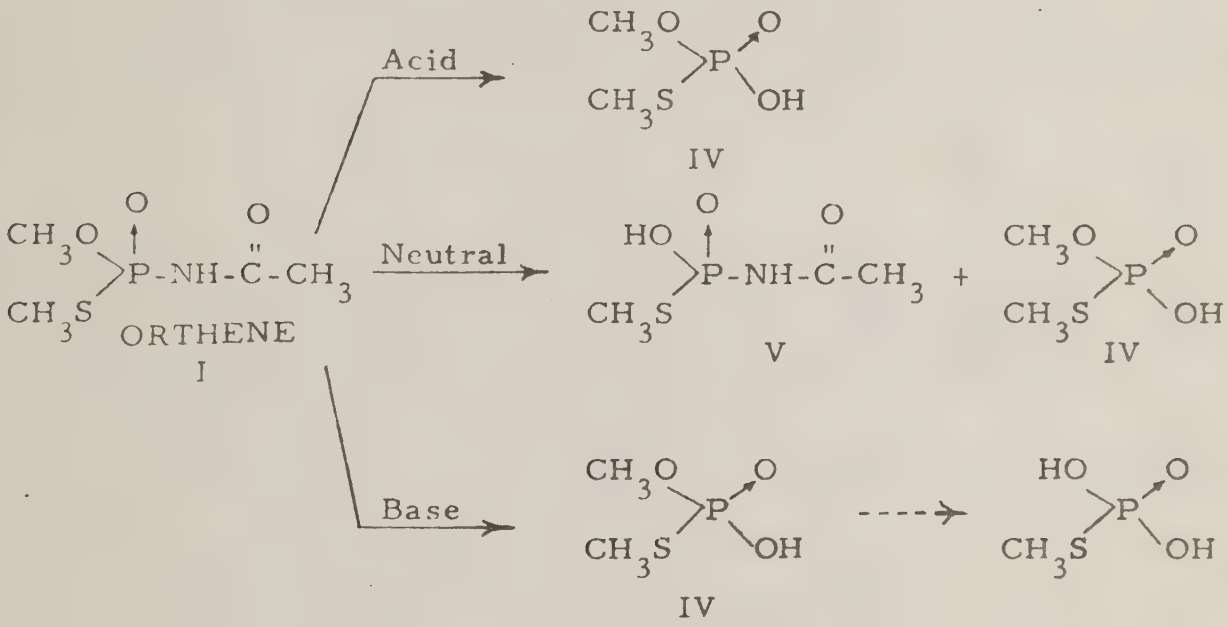
ORTHENE is subject to hydrolysis, but normally at a slow rate (Ref. 4.1). The rate is dependent upon pH and temperatures, the rate being highest at basic pH's. Half-lives at various temperatures and pH's are given in Table VIII. Also given in Table VIII are the equivalent half-lives for ORTHO 9006, which itself is also subject to hydrolysis (Ref. 4.1, 4.2, 4.3).

TABLE VIII

The Half-Lives (in Days) for ORTHENE and ORTHO 9006 in Aqueous Solution as a Function of pH and Temperature

	ORTHENE		ORTHO 9006	
pH	21°C	40°C	21°C	40°C
3	65.5	29.4	22.0	8.4
5	55.2	29.7	107.8	45.1
7	46.4	16.5	44.0	9.8
9	16.1	2.5	9.2	4.8

The major product of hydrolysis of ORTHENE is O,S-dimethyl phosphorothioate (IV). In the physiological pH range, ORTHO 9006 is not formed. At the extreme pH of 3 and 9 traces may be formed. However, it is itself hydrolyzed to IV, which in turn is stable under acid conditions, but degrades slowly under basic conditions (Ref. 4.4). At pH 7, S-methyl acetylphosphoramidothioate (V) is also formed. The hydrolytic route of ORTHENE can be shown by the following diagram.



Ground Water

ORTHENE and ORTHO 9006 move readily in soil with water. However, because they are rapidly degraded in soil, they would not be expected to exist long enough in soil to have time to move into underground water. To determine the actual situation under field conditions, a test was conducted in a sandy soil in Florida (Ref. 4.6). This is an extreme case as the soil is porous and the rainfall heavy. A plot in which lettuce was being cultivated was treated several times with 1 lb ORTHENE per acre. After each rainfall (both natural and artificial, when necessary), ground water samples at several depths, including below the water table (about 4-5 ft. in this test plot) were collected and analyzed. Three months later, the same plot, but without the crop, was treated at 3 lb/acre (3 times the label recommended rate) and water and soil samples taken.

The amount of ORTHENE found in the water taken at 1 ft. varies from 0 for the vast majority of the samples to about 2 ppm (for a 3 lb/acre application with samples taken 0-4 hours after treatment). ORTHO 9006 was only detected at a maximum of 0.06 ppm (this again in the 3 lb/acre - 0-4 hour sample).

ORTHENE was also detected in the soil, but only at the shallower depths. The maximum occurred in the 6-12 inch sample. Traces of ORTHO 9006 (maximum 0.04 ppm) were also seen in the 6-12 inch sample.

No ORTHENE or ORTHO 9006 was detected in any sample of water or soil taken at 2-1/2 ft. or deeper. This is true even after 7 treatments and about 12 inches of rain. Thus, even in this extreme case, neither ORTHENE nor ORTHO 9006 is likely to get into ground water.

Runoff Water

Two tests were conducted to determine if ORTHENE and ORTHO 9006 would be moved by runoff water (Ref. 4.8, 4.9). Using standard practices, plots were cultivated with lettuce. At about half maturity of the crop, the soil between the rows was compacted in such a way that water in each row had a net flow to one end of the plot. At that end a catch pan was arranged common to all rows. ORTHENE was sprayed at 1 lb/acre. This was immediately followed with overhead irrigation with 2 inches of water to create runoff. The runoff water was collected in 3 fractions (first 20%, middle 60% and last 20%). The water and associated soil particles were separated and each was analyzed. One week later the plots were resprayed and after 3 days the irrigation and water and soil collections were repeated.

The data are summarized in Table IX.

TABLE IX

Residues in Runoff Water and Soil
ORTHENE Treatment at 1 Lb/Acre
2 Inches of "Rain" by Overhead Irrigation

Soil-Type Test No. Location	Sample		Residue Found PPM(1)			
			0 Days(2)		3 Days(2)	
	Type	Fraction	ORTHENE	9006	ORTHENE	9006
Loamy Sand T-2290 Florida(3)	Runoff Water	First 20%	0.06	0.00	0.00	0.00
		Middle 60%	0.09	0.00	0.00	0.00
		Last 20%	0.08	0.00	0.00	0.00
	Eroded Soil	First 20%	0.10	0.00	0.02	0.00
		Middle 60%	0.10	0.00	0.02	0.00
		Last 20%	0.13	0.00	0.02	0.00
Loam T-2291 California	Runoff Water	First 20%	0.04	0.00	0.32	0.00
		Middle 60%	0.04	0.00	0.16	0.00
		Last 20%	0.00	0.00	0.10	0.00
	Eroded Soil	First 20%	0.02	0.00	0.16	0.00
		Middle 60%	0.14	0.00	0.07	0.00
		Last 20%	0.00	0.00	0.00	0.00

(1) Averages of duplicate plots.

(2) Interval from treatment to irrigation.

(3) In test T-2290, 0.6 inches of rain fell between the second treatment and the 3-day irrigation.

Small, but significant residues of ORTHENE were found in both the runoff water and the associated soil particles. No ORTHO 9006 was detected in any sample at any time.

In one test (T-2290) 0.6 inches of rain fell between the second application and the 3-day irrigation. Any runoff water from this rain was not collected. Runoff water from this treatment contained no ORTHENE and soil only 0.02 ppm, the limit of detection. On the other hand, both water and soil from the other 3-day interval test (where no rain fell) contained significant residues. Apparently the light rain moved the ORTHENE away from the soil surface making it unavailable to the runoff water, which is mainly a process of washing and eroding the surface.

Surface Water

Although ORTHENE is not intended for use directly in bodies of water such as ponds or lakes, such surface waters could become contaminated from runoff or accidental spillage. Thus, a knowledge of the fate of ORTHENE in surface waters is of importance.

To determine this, two tests were conducted (Ref. 4.9, 4.10). The ponds were treated with ORTHENE at 0.1 ppm in the water. At intervals, samples of the water, bottom mud and submerged vegetation were taken for analysis. The results are summarized in Table X.

TABLE X

Pond Residues
Treatment at 0.1 ppm

Test No. Location	Sample	Chemical	Residue - PPM Interval - Days 0	3	9/10	23/24	44/45
T-2293 Florida	Water	ORTHENE	0.08	0.06	0.03	0.00	0.00
			0.10	0.04	0.00	0.00	0.00
		ORTHO 9006	0.00	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00
	Bottom Mud	ORTHENE	0.07	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00
		ORTHO 9006	0.00	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00
	Submerged Vegetation	ORTHENE	0.37	0.84	0.00	0.00	0.00
			0.24	0.27	0.00	0.00	0.00
		ORTHO 9006	0.01	0.06	0.00	0.00	0.00
			0.00	0.02	0.00	0.00	0.00
T-2294 Iowa	Water	ORTHENE	0.11	0.10	0.08	0.04	0.03
			0.14	0.09	0.08	0.04	0.00
		ORTHO 9006	0.00	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00
	Bottom Mud	ORTHENE	0.04	0.06	0.00	0.00	0.00
			0.03	0.04	0.00	0.00	0.00
		ORTHO 9006	0.00	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00
	Submerged Vegetation	ORTHENE	0.18	0.17	0.09	0.04	0.00
			0.52	0.23	0.09	0.04	0.00
		ORTHO 9006	0.00	0.00	0.00	0.00	0.00
			0.00	0.00	0.00	0.00	0.00

The residues of ORTHENE in the water decreased so that they are below 0.1 ppm within a week and essentially zero by 6 weeks. No ORTHO 9006 was detected in the water at any time.

Both bottom mud and submerged vegetation picked up ORTHENE, the concentration in the vegetation being higher than that in the mud. A small amount of ORTHO 9006 is seen in the vegetation in one test, but none in the mud. These residues rapidly degraded.

Thus, even if surface waters were to become contaminated with ORTHENE, within a short time no residues would remain either in the water or in the mud or vegetation.

FATE IN ANIMALS (Section 5)

The metabolism of ORTHENE has been studied in the rat (Ref. 5.1), the goat (Ref. 5.2) and quail (Ref. 5.3). Also the metabolism of its plant and soil metabolite ORTHO 9006 has been studied in the rat (Ref. 5.4) and goat (Ref. 5.2). Radioactive labelled ORTHENE and ORTHO 9006 were used in all of these studies.

In addition, limited non-radiolabelled studies were conducted in a number of species: meat and milk in the cow (Ref. 5.5), meat in the hog (Ref. 5.6), and meat and eggs in the chicken (Ref. 5.7) and quail (Ref. 5.8).

The metabolism in all species tested is essentially similar. Excretion is rapid and essentially complete. Most of the excretion took place in the first 12 hours and only a low level of elimination was observed thereafter. The main route of excretion is in the urine of mammals and feces of birds. Most of the remaining dose was found in the breath (measured in the rat and quail ^{14}C tests only), feces and traces in the milk (of the cow and goat) or eggs (of the quail and chicken). Only traces of the applied dose were detected in the tissues during dosing and declined rapidly to zero when dosing ceased. None of the body residues was concentrated in any one organ or tissue.

In the mammalian ^{14}C experiments, the radioactivity in the urine was shown to be about 75% unchanged ORTHENE, with about 5% each of DMPT and RE 17,245 (cf. Section 4). No ORTHO 9006 was detected in either the urine or milk. Thus, this chemical is not a mammalian metabolite.

In birds, small amounts of ORTHO 9006 were detected in the feces and traces in the eggs. The ratio of ORTHO 9006 to ORTHENE found is greater than in the dosing material. Thus, although ORTHENE is excreted mainly unchanged in birds, there does seem to be a small amount of metabolism to ORTHO 9006, in contrast to none in mammals. This ORTHO 9006 is not retained in the body, but is itself either further metabolized or excreted.

Although ORTHO 9006 is not a mammalian metabolite, it is a plant metabolite. Since animals grazing exposed plants could ingest this chemical, its fate in animals is also important. This subject has been discussed in detail in the MONITOR Tolerance Petition (PP 0F0956). In brief, ORTHO 9006 is mostly metabolized to $^{14}CO_2$. Very small traces of unchanged material are excreted slowly in the urine. The remainder of the dosed ^{14}C is retained as incorporated material. There is no retention by the animal body, nor is there any excretion of ORTHO 9006 into the milk.

TOXICITY AND HAZARD TO MAN, WILDLIFE AND OTHER NONTARGET SPECIES (Section 6)

ORTHENE is an extremely weak in vitro inhibitor of acetyl cholinesterase (Ref. 6.1). The molar I_{50} is in excess of 2.7×10^{-3}, the highest concentration tested.

This unique property of ORTHENE is further reflected in the very low acute and subacute toxicity of the chemical to a wide variety of mammals, fish and birds.

A summary of the toxicology tests carried out and the data obtained is given in Ref. 6.2. The original reports are to be found in Section C of the ORTHENE Residue Tolerance Petition. The data for the plant and soil metabolite, ORTHO 9006, can be seen in MONITOR Residue Tolerance Petition PP 0F0956.

From the view point of hazard to wildlife and other nontarget species, the toxicology data for ORTHENE can be reviewed as follows:

It is unlikely that wildlife will be exposed to acute toxic levels of ORTHENE. However, the data obtained in the laboratory show that ORTHENE presents a minimal hazard at this high dosing or exposure level. Table XI summarizes the acute toxicity of ORTHENE to a variety of species.

TABLE XI

The Acute Toxicity of ORTHENE

Species	Type of Exposure	Material	Results
Animal:			
Rat	Oral	Technical	LD_{50} 900 mg/kg
Rat	Oral	Formulated 75S	LD_{50} 1500 mg/kg
Dog	Oral	Technical	MLD 680 mg/kg
Rabbit	Oral	Formulated 75S	LD_{50} 700 mg/kg
Rabbit	Dermal	Formulated 75S	LD_{50} <10,000 mg/kg
Rat	Vapor	Technical	no toxic effects after 4 hours exposure to saturated vapor
Birds:			
Mallard duck (young)	Oral	Technical	LD_{50} 350 mg/kg
Pheasant	Oral	Technical	LD_{50} 140 mg/kg
Fish:	Water	Formulated 75S	96 hr TL_{50}
Most susceptible species tested - Black bass			1,725 ppm
Least susceptible species tested - Goldfish			9,550 ppm
Gambusia		Formulated 75S	6,650 ppm

Laboratory studies have been carried out to evaluate the subacute exposure hazard. The data obtained indicate that the hazard is negligible to animals.

Exposure by Oral Ingestion (Mammals)

In a 90 day feeding study rats showed no significant toxic manifestations other than a slight lowering of the blood and serum cholinesterase activity, at 30 ppm ORTHENE in the diet. At 300 ppm level there was no abnormality in weight gain, food consumption, survival, blood and urological or pathology studies. The same is true for dogs exposed to ORTHENE at the same level in the diet.

Exposure by Physical Contact (Mammals)

Apart from the lowering of RBC cholinesterase levels, there were no overt signs of toxicity from dermal exposure of up to 2 g/kg/day during a 21-day period. Formulated ORTHENE did not sensitize rabbits or guinea pigs although there was evidence of mild skin irritation during exposure. Birds were unaffected by up to 300 mg/kg dermally applied to their legs (simulated perching exposure study).

Exposure by Inhalation (Mammals)

In laboratory studies rats showed no signs of poisoning during a 1 hr/day two week exposure to a 4% aerosol of formulated ORTHENE. This level is the maximum anticipated field concentration for ORTHENE. All other vital signs were identical to unexposed control animals.

Exposure and Effects on Progeny

At 3 mg/kg (the maximum level tested) ORTHENE does not cause teratogenesis in rabbits. The study showed that at this level there were no external, weight or survival abnormalities. A study in which pregnant rats were exposed up to 200 mg/kg ORTHENE, also showed no differences in fetal body weight, external, skeletal or internal development to indicated animals. At this maximum level there was slightly more resorption than in control rats.

ORTHENE does not cause mutagenic or neurotoxic effects in standard mice and chicken studies.

A 3-generation rat reproduction study showed that 30 ppm ORTHENE in the diet was a no effect level. At the 100 and 300 ppm levels mating indices were reduced. A reduction in fertility index occurred at these higher levels in the second generation but not in the third generation. Other parameters of reproduction were essentially the same for the test and control groups. Number of pups and survival rate were reduced at the higher feeding levels in the second generation but not in the first and third generations. During the final generation, as in the first generation, the population data as well as pup survival indices revealed no differences between test and control groups which could be correlated with ingestion of ORTHENE.

Birds exposed to a maximum of 30 ppm in the diet showed no significant pathological or mortality differences to untreated birds. The viability of the chicks from hens exposed to 30 ppm was lower than the control.

laboratory trial. actual field (orchard) trials by Johnson of WA. State U. shows ORTHENE ... bees / Horticulturist / Scientist

in fact ... same companies

Toxicity to Beneficial Insects

ORTHENE is toxic to bees present at the time of application. However, in a comparative test with worker honey bees, ORTHENE was less toxic than malathion, but slightly more toxic than carbaryl (Ref. 6.2).

Tests were conducted to determine the relative toxicity of ORTHENE, carbaryl, parathion and diazinon to the following species of beneficial insects (Ref. 6.3):

Species	Type	Stage	Usual Host (s)
Tachinaephagus zelandicus	Wasp	Adult	Housefly pupae, certain parasitic wasps
Chelonus blackburni	Wasp	Adult	Pink bollworm
Chrysopa carnea	Lacewing ("aphid lion")	Larva	Aphids, mites and several soft-bodied insect species and insect eggs
Muscidifurax raptor	Wasp	Adult	Housefly pupae
Hippodamia convergans	Ladybug	Adult	Aphids, mites, scale insects, soft-bodied insect species

ORTHENE and parathion were each tested on all 5 species; diazinon and carbaryl were each evaluated on 3 species.

ORTHENE was virtually inactive against 2 of the 5 species tested (Chelonus, Chrysopa), whereas parathion, diazinon and carbaryl caused mild-to-strong mortality on these same 2 species. In the case of 2 other species (Muscidifurax, Hippodamia) ORTHENE was fairly toxic, but overall was less toxic than parathion or diazinon. Carbaryl was virtually inactive on Muscidifurax and was not tested on Hippodamia. ORTHENE showed strongest toxicity against Tachinaephagus being about equal to parathion and more active than either carbaryl or diazinon. The latter two materials caused medium-to-strong mortality on Tachinaephagus.

It is clear that ORTHENE has little toxic effect on Chelonus and Chrysopa, and that its toxicity to Hippodamia is less than parathion. This suggests that ORTHENE may be a useful tool in integrated insect control programs.

Toxicity to Soil Microorganisms

A test was conducted by Dr. Dennis Focht, Soil Microbiologist at the University of California, Riverside, California, to determine the effect of ORTHENE and ORTHO 9006 on soil microorganisms (Ref. 6.4).

A Hanford loamy sand, a Domino silt loam and an Altamont clay loam were treated separately with three repeated applications (20 ppm) of ORTHENE and ORTHO 9006 over a 50-day time span. There was no difference in fungal, bacterial, or actinomycete populations between treatments and control, nor could any effect be shown by either treatment on respiration, ammonification, nitrification and sulfur oxidation rates within the soils. Nitrate and ammonium levels remained constant throughout. The higher sulfate levels in the treated soils are attributed to degradation of the added substrate to sulfate. Replica plating failed to isolate any bacteria from untreated soil that were adversely affected by either ORTHENE or ORTHO 9006.

Thus, neither ORTHENE nor its metabolite ORTHO 9006 has any effect upon soil microorganism populations or metabolic processes, even when used repeatedly at rates several times the label recommended rate.

Exposure by Humans

A monitoring and medical study was done on several men occupationally exposed to ORTHENE in a pilot plant where ORTHENE was being produced or in a formulation lab where large batches of ORTHENE were formulated (Ref. 6.5). The men performed their regularly assigned duties. Their urine was monitored for ORTHENE and related chemicals and complete medical studies were made. Although concentrations of up to 5 ppm ORTHENE were found in their urine, thus indicating exposure to ORTHENE, none showed any effect on their health status. There was no effect on their blood cholinesterase levels, one of the most sensitive indicators of organophosphate exposure.

In a second study, field research workers were monitored (Ref. 6.6). Analysis of their urine also showed exposure to ORTHENE, but to a much lesser extent than those in the pilot plant study.

Cotton field plots were sampled to determine potential exposure of field workers to ORTHENE residues (Ref. 6.7). Plots were treated at 1 or 2 lb/acre with 5 to 8 weekly applications. Leaf samples showed that wiping approximately one hour after aqueous spray treatment removed up to 30% of the ORTHENE contained in or on the leaves. Twenty-four hours later (leaves wet with morning dew) the percent removed with wiping did not appreciably decrease, but the total or actual amounts so removed declined by 50%. Gloves and shirt sleeves worn by workers hand harvesting treated cotton showed a sharp decline in residues with interval of reentry time exposure. In the seventh day after the last treatment, there was a ten fold decrease in residues on the gloves. Residues of the plant metabolite, ORTHO 9006, in the gloves and sleeves similarly declined.

Over 80 persons have been involved in field development programs using ORTHENE (Ref. 6.2). To date there have been no problems of intoxication

Thus, when handled properly ORTHENE poses no health hazard to persons producing, formulating, spraying, or working in sprayed fields.

Summary of Effects on Nontarget Organisms

From the above data it can be seen that ORTHENE is of low toxicity to nontarget organisms. This fact, along with its rapid degradation in the environment, indicates that ORTHENE when used according to label directions, poses little or no threat or hazard to nontarget organisms.

FOOD CHAIN (Section 7)

Studies have been conducted to determine if ORTHENE is bioconcentrated in the food chain. Isolated species tested include a diatom, a water flea, an earthworm, a fish, mammals and birds. The fate of ORTHENE in a Model Ecosystem was also determined.

Diatoms

In the first test (Ref. 7.1), a marine diatom (Cylindrotheca fusiformis) was used. The diatom was grown for one week in solutions of ORTHENE at concentrations of 1, 10 and 100 ppm. The metabolite ORTHO 9006 was also tested at 1 and 10 ppm. Neither chemical bioconcentrated to any extent at any level of treatment. Under identical conditions DDT bioconcentrated 1000 to 7000 fold.

Water Flea

A fresh water flea (Daphnia magnus) was also tested (Ref. 7.2). Adult Daphnia were treated with both ORTHENE and ORTHO 9006 for several days. Neither bioconcentrated, whereas under identical conditions DDT bioconcentrated over 500 fold.

Earthworms

Earthworms were also tested (Ref. 7.3). The worms were kept in soil treated with ORTHENE at the equivalent of 2 lb/acre and ORTHO 9006 at 0.1 to 0.8 lb/acre In both chemicals, the concentration of the chemical in the worm was 1/2 to 1/8th the concentration in the soil. The residues in the worms disappeared when the worms were transferred to untreated soil. Thus, no bioconcentration occurs in earthworms and what residues do occur are rapidly eliminated. Also, there was no metabolism of ORTHENE to ORTHO 9006 in the worm.

Fish

Bluegill sunfish were continuously exposed to 1.0 mg/l or 0.01 mg/l ORTHENE for 35 days and tissue samples analyzed periodically to investigate rate and extent of ^{14}C-residue during exposure (Ref. 7.4). After the exposure period, fish were transferred to an uncontaminated system for 14 days and samples analyzed periodically to investigate residue decline.

During the 35-day exposure period, no mortality was observed. The fish in all experimental units appeared normal, fed readily, and were judged to be in excellent physical condition.

Based on the results of radiometric analyses of fish tissue samples the following conclusions are evident:

1. A dose-response relationship exists, that is, as exposure levels increase, ^{14}C-residues in edible portion of fish increase.

2. The maximum tissue concentration of ^{14}C-residue in the edible portion is only ca 10X the concentration of residue in water.

3. Upon transfer to uncontaminated water, fish exposed to both levels of ORTHENE eliminated more than 50% of the ^{14}C-residues present in the edible portion within 3 days.

In view of the extremely low bioconcentration factor observed for ORTHENE, it is concluded that the environmental hazard associated with accumulation of harmful residues in fish is minimal.

Mammals

To determine if ORTHENE and/or ORTHO 9006 could be transferred to the young in nursing mammals, cows and goats were dosed with ORTHENE, ORTHO 9006 and mixtures (Ref. 5.2, 5.5). During dosing with ORTHENE, small amounts of ORTHENE appeared in the milk. Forty-eight hours after dosing, the amount in the milk decreased to 0.01 ppm or below. No ORTHO 9006 was detected in the milk from dosing either with ORTHENE or with ORTHO 9006 itself.

Thus, although a small proportion of the ingested ORTHENE is found in the milk, there is no concentration in the milk (like that which occurs with DDT). Also, when the exposure stops, no further excretion into the milk occurs.

ORTHENE and ORTHO 9006 were not concentrated in the flesh or tissues including fat of mammals (Ref. 5.1, 5.2, 5.4, 5.5, 5.6), either during dosing or after cessation of dosing. Thus, there will be no bioconcentration from the tissues of herbivores to carnivores.

Birds

ORTHENE and ORTHO 9006 do not concentrate in the flesh, tissues (including fat), or eggs of birds (Ref. 5.3, 5.7, 5.8).

Model Ecosystem

The fate of ORTHENE in the Model Ecosystem developed by Dr. Robert Metcalf and his associates was studied by Dr. Gary Booth (Ref. 7.5). Dr. Booth's conclusions are:

Under the conditions of these experiments, it is concluded that ORTHENE will not persist as a chemical pollutant in the environment. The basic summary and conclusions of these experiments can be summarized as follows:

1. ORTHENE was found only in water samples.

2. ORTHENE was mainly metabolized to ^{14}C "fragments" which were naturally incorporated into constituents of living tissues.

3. The known metabolites, ORTHO 9006, DMPT and RE 17,245 did not accumulate or concentrate in any of the tissues.

4. Very few unknown materials were detected. The major portion of them (0-37.6%) were highly polar (water soluble) materials which would not accumulate in lipids.

5. The small amounts of RE 17,245 and ORTHO 9006 that were detected were found only in hydrolyzed water samples suggesting that these chemicals are likely conjugated during the cycling of the Ecosystem and, hence, rendering them harmless to living tissues.

6. The complete lack of residues in the fish, the organism at the top of the food chain (in this system) suggests that ORTHENE could be used favorably around aquatic ecosystems.

7. ORTHENE does not show any indications of accumulating in living tissues. Thus, ORTHENE and its metabolites do not pose serious threats as biological magnifiers.

Bioconcentration Summary

Neither ORTHENE nor its metabolite ORTHO 9006 bioconcentrates in any organism tested from single celled diatoms to the most advanced organisms; mammals and birds. Thus, ORTHENE and its metabolites do not pose any threats as biological magnifiers.

APPENDIX E

SPRUCE BUDWORM PILOT TEST OF TRICHLORFON (DYLOX) AND CARBARYL (SEVIN-4-OIL): I. The impact on breeding bird numbers and nesting success[1]

Lawrence R. DeWeese[2] and Charles J. Henny[3]

INTRODUCTION

The Beaverhead National Forest in southwestern Montana was chosen as the 1975 site to pilot test trichlorfon (Dylox)[4] and carbaryl (Sevin-4-oil)[4] for Western spruce budworm control at 1 lb/acre (active ingredient). The Section of Pesticide-Wildlife Ecology of the Denver Wildlife Research Center was contracted by the U.S. Forest Service, Region 1 Office to evaluate the impact of the spray program on bird populations. This report briefly presents the objectives, methods, and some preliminary results from the bird studies.

To minimize the exclusion of significant phenomena, several methods were used to detect and quantify direct and indirect effects of the

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[1]Results incomplete and not for publication or use without authority of the Director, Denver Wildlife Research Center.

[2]U.S. Fish and Wildlife Service, Denver Wildlife Research Center, P. O. Box C, Davis, California 95616.

[3]U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Bldg. 16, Federal Center, Denver, Colorado 80225.

[4]Reference to trade names does not imply U.S. Government endorsement of commercial products.

aerial applications of the two insecticides on resident birds. Stickel (1974) pointed out that there are two primary techniques for studying field applications of phosphates and carbamates: (1) a careful search for sick or dead birds and (2) the analysis of brain or blood for cholinesterase inhibition. Stickel did not suggest counts of living birds because of a possible temporary exodus, simply as a result of reduction in food supply. Our study utilizes the two approaches recommended by Stickel but also includes (3) the study of live birds [illegible] census techniques, (4) the determination of nesting success (an index) at as many nests as possible, and (5) the exploration of the food habits of resident birds as they relate to the spruce budworm and other important insects. We realize that birds may leave an area due to temporary loss of food supply, but we wanted to evaluate the magnitude of such a temporary loss if it occurred. Furthermore, the live bird census information could possibly aid in interpreting reduced nesting success if it occurred in the treatment plots. Preliminary results regarding the density and species composition of breeding pairs before and after spray and the determination of reproductive performance are presented in this interim report. Brain cholinesterase information is included in part II of this study. Other data are not yet analyzed, but will be included in a final report to be submitted to a proper journal for publication.

METHODS

Details of the major plot locations, dates sprayed, application rates, formulation of the insecticides and operational summaries are not

given here. These data will be prepared by the USFS. Briefly, among nine 1-2,000-acre plots three were sprayed by helicopter with Sevin-4-oil formulation, three with a Dylox formulation and three were untreated. Each was a single application made in early morning at a calculated rate of 1 lb/acre (active ingredient).

An important aspect of our study was to locate, map, and revisit all nests that we could find. Visits to nest sites were minimized to reduce effects of human intrusion and disturbance; however, important events, such as nest building, egg laying, number of eggs laid, number of young hatched and fledged were recorded. A cavity viewing device (DeWeese et al. 1975) and dental inspection mirror were used for viewing into cavity nests. An end-mounted mirror on a telescoping pole was used for observation of open-type nests.

For breeding pair censuses, we established a 20-acre rectangular subplot within each of nine major plots that the USFS had chosen for the test. The dominant criterion for these subplots was to have similar habitats which would likely yield similar bird communities. Each subplot was oriented such that the 1,320-foot side generally crossed a major drainage at nearly a right angle and the 660-foot side paralleled the drainage. All subplots contained a drainage. Forested habitat available for bird studies in the original major plots was dominated by Douglas-fir (Pseudotsuga menziesii) habitat types as described in Pfister et al. (1974). Overall treatment boundaries were then modified somewhat for our subplots to include additional bottom lands in the major drainages associated with each major plot. Boundaries were marked

so that untreated areas were not less than one-quarter mile from our subplots. All subplots included a variable percentage of the three common habitats found in the 6-8,000-foot elevational range in this area. These habitats as labelled by their dominant overstory were: (1) Douglas-fir, (2) aspen (Populus tremuloides), and (3) big sagebrush (Artemisia tridentata). Other occurring habitats included open grassy meadows and willows associated with the stream-bottom complex, as well as understory complexes in association with habitats described here.

An internal, lettered-numbered grid of stakes was surveyed into the subplots as described in Pillmore (1973:145). We assigned plots and data collection routines to three people experienced in field ornithology and made some duplicate counts ourselves to evaluate their coverage. Each person was assigned to three plots, each with different treatments, for the entire study, mornings were spent censusing, and other data were collected during afternoons. Trial censuses were made to familiarize personnel with procedures and the birds and we began collecting data by mid-June and continued through July. All data were logged daily into a separate notebook for each plot.

Five estimates of breeding pairs were made during 3-week periods before and after the insecticide application. Each breeding bird census began at official sunrise and extended for two hours. Breeding pairs were mapped by species as presented by Svensson et al. (1970). In addition, other information, such as weather, bird behavior, nest locations and occurrence of dead birds on the plots was also recorded. Effort and results of searching for dead or sick birds were specifically quantified on all plots throughout the study.

We will briefly mention the methods used for fixed-station counts, however, results are not available at this time. Three to five round-shaped, fixed-stations were flag-marked at their periphery and central spot in each of the major habitats. Size of the stations varied from one-third of an acre to three acres, depending upon availability of habitat, but their adjacent boundaries were never less than 100 yards apart. A route of stations was established apart from the 20-acre subplots in each major plot. Station counts for 5 minutes were made at the central spot after a 1-minute initial pause at each successive station. This count routine was performed after the breeding bird censuses each day from two hours after sunrise until completion. This fixed-station method of counting forest breeding birds is not a standard practice; the method described here is a synthesis of our own design with comments and assistance from Chandler S. Robbins (personal communication). Time at completion varied from 11:00 a.m. to 11:45 a.m., depending on the plot, but was fairly consistent among counts on the same plot. Bird species were segregated by their occurrence inside and outside the fixed-station boundary and by sex, when known. These data represent an index to changes in bird numbers and species composition after treatments by acreage and time for each habitat.

Food items were excised from stomachs of 150 birds shot for brain samples. These plant and animal food materials will be sorted out, identified and tabulated for each bird. The stomach contents are now in a preservative and awaiting more refined identification.

RESULTS AND DISCUSSION

Nesting Success

Nests that were active at spray time are presented in Table 1. Species that nested in cavities comprised 56% and those not in cavities comprised 44% of the nests. Thrushes (22%), the woodpecker group (17%), flycatchers and swallows (17%), the house wren (14%), sparrows and juncos (11%) and the warbling vireo (7%) comprised the majority of the nests. An additional 73 nests were initially observed but the adults had terminated their nesting activities before the plots were sprayed. Species are also classed by their general feeding strategy. In this way, species with similar food-gathering habits and with similar potentials for insecticide exposure are grouped to increase the sensitivity of our comparisons. Inspection of stomach contents from several species perhaps will necessitate a more meaningful species grouping.

Outcome of nests that were active at treatment time is an important indication of the overall success of breeding birds in treatment and nontreatment areas. Nest results within treatment groups, by nest type and also by feeding strategies are shown in Table 2. We must emphasize that our nesting success data are indices. Nests active at spray time were well on their way to a successful outcome since the pressures of desertion and nest loss during early nesting had already been exerted, thus, the success indices are quite high. The percentage of nests active at spray time which were ultimately successful held consistent during the postspray period regardless of treatment. In the control plots, 74% of nests with eggs and 97% of nests with young at spray time were successful, in the Dylox plots 90% and 100% were successful, and in the Sevin-4-oil

plots 86% and 100% were successful. These data suggest that the nesting process of species for which we found nests was not generally disrupted by the insecticide treatments.

We attempted to determine the outcome of all known active nests but could not do so in many cases. A weekly summary of the final visits made after spraying to the nests that were active at spray time is shown in Table 3. Those data indicate that we made a similar effort to recheck nests in all plots.

Breeding Pair Estimates

A schedule of breeding bird censuses that permitted daily bird counts on plots with different treatments was followed as shown in Table 4. Given the spray schedule of 1 plot/day, we patterned postspray counts so that all plots were censused nearly the same number of times, and on days with equal time elapsed since treatment. Census data from the control plots were divided into two periods similar to the pre- and post-treatment periods for the sprayed plots.

Many different species of birds inhabited our 20-acre subplots. Although some observed differences in occurrence and abundance of a few species were apparent among plots, the more abundant species occurred on all plots (Table 5). Twenty of the 34 common species that were abundant enough and met the requirements of our census are shown. An additional two species (evening grosbeak and pine siskin) were obviously abundant but their behavior and territorial traits prevented a meaningful census of their breeding pairs. Also, 16 other species occurred either uncommonly

or their breeding status was unknown in the subplots; 20 more species we uncommon and registered on treatment plots only, 5 on control plots only and 5 on control or treatment plots. In all, 66 species were encountere during the breeding pair censuses.

When grouped by feeding strategies (Table 6), breeding pair estimat showed no decrease or increase after treatments unique to the sprayed plots. Species were grouped into the five feeding strategies that were also used for nesting outcomes. Total breeding pairs changed by more tha 20% after treatment in those groups with greater than 20 prespray pairs for (1) aerial feeders in control, (2) aerial and tree-canopy feeders in Dylox, and (3) no groups in Sevin-4-oil-treated areas. The total breedin pair estimate for the postspray period was 91% of the prespray estimate i the control plots, 88% of the prespray estimate in the Dylox plots, and 92% of the prespray estimate in the Sevin-4-oil plots.

Our breeding pair estimates as presented here may be influenced by many factors including insecticide exposure. Great concern must be voiced when large differences in the breeding pair density occur or a species becomes completely absent after treatment. This was not noted in our study.

Casualty Searches

Two search efforts were made for dead or sick birds in all plots throughout the study. The first, and most important, was specifically to look for dead or sick birds while doing nothing else. Results of these searches (Table 7) clearly indicate that mortalities did not increase after treatment on treated plots. Also, in support of this finding, is a secondary effort that each observer made while walking many hours on

constant routes to, from, and during routine bird censuses. We found a few dead birds on the census routes in about equal frequencies on all plots. No sick birds were found during any search efforts which further suggests that mortalities that we encountered were likely not insecticide induced.

SUMMARY

Objectives, methods and preliminary results of effects of aerial applications of Sevin-4-oil and Dylox (both 1 lb/acre [active ingredient]) on birds in a pilot test for controlling Western spruce budworm are presented. Results from nest monitoring and breeding pair estimates are given; results of cholinesterase studies are presented as a separate report. Two additional sets of information will be included in a final report for publication.

Outcome of observed nests and estimates of breeding bird density and diversity showed similar patterns on control and treated plots after treatment. Searches for sick or dead birds showed no increase in mortalities on treated plots. Mortalities encountered during specific searches were likely not insecticide induced. The results support a conclusion that immediate adverse effects on birds, if any, were not obvious from the standpoint of the described approaches. Statistical comparisons of the data are not given in this preliminary report, pending further review of these and additional data. Literature from previous studies will also be included in the final report.

ACKNOWLEDGMENTS

Three summer employees deserve special recognition for their outstanding efforts in collecting much of the basic information used in this report. Kathie A. Bobal, Randy L. Floyd, and Albert W. Shultz were in the field at sunrise most of the summer to observe the birds during their peak activity period--their efforts are most appreciated.

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Table 1. Number and type of nests and general feeding strategies of breeding bird species found active on the study areas at the time of spray.

Species	Nest Type[a]	General Feeding Strategy[b]	Nests Active at Spray		
			Control	Dylox	Sevin-4-oil
Goshawk[c]	Non-cavity	Raptorial	0	1	0
Sharp-shinned Hawk	Non-cavity	Raptorial	1	0	0
Red-tailed Hawk	Non-cavity	Raptorial	1	1	1
Common Flicker	Cavity	Ground	3	4	6
Yellow-bellied Sapsucker	Cavity	Tree-trunk	7	6	4
Williamson's Sapsucker	Cavity	Tree-trunk	0	1	0
Hairy Woodpecker	Cavity	Tree-trunk	2	2	2
Downy Woodpecker	Cavity	Tree-trunk	1	2	1
Northern Three-toed Woodpecker	Cavity	Tree-trunk	0	0	1
Empidonax spp. Flycatcher	Non-cavity	Aerial	4	7	3
Tree Swallow	Cavity	Aerial	6	18	3
Mountain Chickadee	Cavity	Tree-canopy	3	7	5
Black-capped Chickadee	Cavity	Tree-canopy	1	0	0
House Wren	Cavity	Understory	10	14	11
American Robin	Non-cavity	Ground	14	8	13
Swainson's Thrush	Non-cavity	Ground	0	0	2
Mountain Bluebird	Cavity	Air-ground	6	7	3
Warbling Vireo	Non-cavity	Tree-canopy	7	4	5
Yellow Warbler	Non-cavity	Understory	0	1	0
Yellow-rumped Warbler	Non-cavity	Tree-canopy	0	1	0
Pine Siskin	Non-cavity	Tree-canopy	0	2	0
Western Tanager	Non-cavity	Tree-canopy	0	0	2
Green-tailed Towhee	Non-cavity	Understory	2	0	0
Dark-eyed Junco	Non-cavity	Ground	3	7	3
Chipping Sparrow	Non-cavity	Ground	5	5	3
White-crowned Sparrow	Non-cavity	Ground	1	1	0
		Totals	77	99	68

[a] Based on nest sites utilized in the study area.

[b] Based on personal experience and observations during this study; also from Smithsonian Institution U.S. Natl. Mus. Bulletins on Life Histories of Birds of North America by Arthur C. Bent.

[c] Scientific names of birds are found in Table 8.

Table 2. Results of nesting as determined at the last postspray visit to nests that were active at the time of spray[a].

Treatment	Nests With Eggs at Spray Time[b]					Nests With Young at Spray Time[b]			
			Percentage of Nests With Known Outcome				Percentage of Nests With Known Outcome		
	No. Species	No. Nests	Active or Fledged[c]	Failed[d]	Total Unknown	No. Nests	Active or Fledged[c]	Failed[d]	Total Unknown
CONTROL									
Nest Type									
Cavity	9	16	73	27	1	19	100	0	1
Non-cavity	13	13	75	25	1	14	92	8	2
Total	22	29	74[g]	26[g]	2	33	97[g]	3[g]	3
Feeding Strategy[e]									
Raptorial	2	1	100	0	0	1	0	0	1
Aerial	2	7	29	71	0	3	100	0	0
Tree-canopy	3	0	0	0	0	3	100	0	0
Tree-trunk	3	2	100	0	0	7	100	0	0
Ground	7	8	86	14	1	14	100	0	2
Understory	4	8	100	0	0	3	67	33	0
Air-ground	1	3	50	50	1	2	100	0	0
Total	22	29	74[g]	26[g]	2	33	97[g]	3[g]	3

Table 2 (cont'd)

DYLOX									
Nest Type									
Cavity	9	19	100	0	4	33	100	0	6
Non-cavity	14	19	78	22	5	10	100	0	1
Total	23	38	90[g]	10[g]	9	43	100[g]	0	7
Feeding Strategy[e,f]									
Raptorial	3	0	0	0	0	3	100	0	0
Aerial	2	18	100	0	4	1	100	0	0
Tree-canopy	3	0	0	0	0	9	100	0	2
Tree-trunk	4	0	0	0	0	10	100	0	2
Ground	7	11	67	33	2	7	100	0	0
Understory	3	7	100	0	1	9	100	0	2
Air-ground	1	2	0	0	2	4	100	0	1
Total	23	38	90[g]	10[g]	9	43	100[g]	0	7
SEVIN-4-OIL									
Nest Type									
Cavity	9	13	91	9	2	18	100	0	3
Non-cavity	10	12	80	20	2	7	100	0	1
Total	19	25	86[g]	14[g]	4	25	100[g]	0	4

Table 2 (cont'd)

Feeding Strategy[e]									
Raptorial	1	0	0	0	0	1	100	0	0
Aerial	2	4	100	0	0	1	100	0	0
Tree-canopy	3	2	100	0	0	4	100	0	0
Tree-trunk	4	0	0	0	0	7	100	0	1
Ground	5	10	75	25	2	9	100	0	2
Understory	3	7	100	0	2	2	100	0	0
Air-ground	1	2	50	50	0	1	0	0	1
Total	19	25	86[g]	14[g]	4	25	100[g]	0	4

[a]See Table 3 for the frequency of final visits made to nests active at spray time.

[b]An additional 8 nests of 4 species in control plots that built nests at spraying or thereafter had 1 failure, 4 active or fledged and 3 unknown outcomes at the last postspray check; 9 similar nests of 4 species in Dylox plots had 3, 5, and 1, respectively, at last check, and 6 nests of 4 species had 0, 5, and 1, respectively, in Sevin-4-oil-treated plots.

[c]Active nests include those with eggs or young or with adults tending the unseen contents; "fledged" nests produced at least 1 young to the age of leaving the nests; young were detected by visual and auditory techniques.

[d]Causes of failures included disturbances from predators, weather, livestock, humans, and unknown factors.

[e]The majority of Warbling Vireo nests that were active at spray time are not included because we could not see into the nests; of 8 additional nests on control plots, 7 were active and 1 had unknown status; 1 nest on Dylox was active, and of 3 nests in Sevin-4-oil-treated plots, 2 were active and 1 had unknown status at the last postspray check.

[f]Stellar's Jay (1 nest), not included in table, had unknown outcome postspray.

[g]Weighted mean percentage.

Table 3. Weekly summary of final postspray visits to nests that were active at spray time.

Treatment	Numbers of Nests at Weekly Intervals Postspray: Nesting Not Terminated[a] 1	2	3	4	Nesting Terminated[b] 1	2	3	4	Result Unknown	Total Nests
Control	6	13	10	2	6	15	5	7	13	77
Dylox	2	13	19	0	20[c]	11	13	0	21	99
Sevin-4-oil	6	10	1	5	9	16	3	4	14	68

[a]Nests not terminated at final visit were treated as active in nest studies.

[b]All terminated nests were categorized by their results; see Table 2.

[c]Proportionally, more early nesters were found in the Dylox plots; see Table 2 for percentage nesting successfully.

Table 4. Schedule of breeding-bird, fixed-station counts and treatments on the nine study plots.[a]

Date	Plots Pre-Spray 1	2	3	4	5	6	7	8	9	Date	Plots Post-Spray 1	2	3	4	5	6	7	8	9
June 15		C[b]			C				C	July 8									
16	C					C	C			9									
17			C	C				C		10		T							
18		C								11		C			C	T			C
19	End Trial Counts--Begin Counts									12	C					C	C	T	
20		C			C				C	13		C	C					C	
21	C					C	C			14				T		C			C
22			C	C				C		15				C				C	
23		C			C				C	16	C		C		T				
24	C									17		C			C		T		C
25						C	C			18				C		C	C		
26			C	C				C		19	C				C			C	
27		C			C				C	20		C	C				C		
28	C					C	C			21				C		C			C
29			C	C				C		22	C				C			C	
30										23		C	C				C		
July 1		C			C				C	24						C			C
2	C					C	C			25				C	C			C	
3			C	C				C		26	C								
4		C			C				C	27		C	C				C		
5	C					C	C			28				C		C			
6			C	C				C		29					C			C	
7										30			C				C		

a Plots 1, 4 and 2 were assigned to first observer, plots 3, 5 and 6 to a second, and 9, 7 and 8 to a third observer; treatments within each set of plots were control, Dylox and Sevin-4-Oil respectively.

b C=date breeding-bird and fixed-station counts were made; T=date a plot was treated.

Table 5. Estimated pairs of selected breeding birds in the nine plots with respective treatments during the pre- and post-spray periods.[a]

Species[b]	Overall Rank[c] Pre-Spray	Control Plots						Dylox Plots						Sevin-4 Oil Plots					
		Pre-Spray			Post-Spray			Pre-Spray			Post-Spray			Pre-Spray			Post-Spray		
		1	3	9	1	3	9	4	5	7	4	5	7	2	6	8	2	6	8
Common Flicker	14	0	1	1	0	1	1	1	1+	1	1	0	2	P	1+	P	P	2	0
Yellow-bellied Sapsucker	12	2	P	1	2	1	1	1	1	1	0	0	1	1	0	1	1	1+	0
Empidonax Spp.[d]	2	7	5	6	4	4	4	7	4	3	4	3	2	7	6	5	9	4	6
Tree Swallow	10	2	1	1	2	1	1	2	1+	4	2	0	5	1	3	P	0	2	0
Mountain Chickadee	5	2	4	4	2	4	3	4	3	3	3	2	4	3	4	5	2	4	2
House Wren	8	2	2	3	3	2	3	3	2	2	3	1+	2	3	4	0	2	3	0
American Robin	3	8	3	3	5	3	6	6	2	3	8	1+	4	6	8	3	4	6	5
Hermit Thrush	14	0	2	1	0	1	0	P	1	0	0	1	+	1	0	3	1	1	3
Swainson's Thrush	10	4	1	1	3	0	1	1	0	1	1	0	1	2	0	3	2	0	3
Mountain Bluebird	15	1	0	0	1	P	0	1	P	1	0	0	1	0	1+	0	0	0	0
Ruby-crowned Kinglet	7	1	4	4	0	2	3	3	2	P	2	2	P	3	4	3	3	2	2
Warbling Vireo	1	7	3	7	4	3	6	8	5	6	9	3	3	9	6	4	10	6	3
Yellow-rumped Warbler	4	4	4	5	4	4	5	6	3	1	5	1+	P	5	3	4	5	3	3
Macgillivray's Warbler	10	0	0	2	1	1	2	2	2	2	3	2	0	2	0	3	1	0	4
Western Tanager	9	1	3	3	2	3	3	2	2	0	0	2	0	5	2	3	5	3	3
Lazuli Bunting	10	3	0	1	5	0	3	1	2	0	3	3	0	4	2	2	4	2	3
Cassin's Finch	13	P	P	1	0	0	0	2	P	P	1	0	2	2	2	0	1	2	0
Dark-eyed Junco	5	4	4	4	4	6	2	6	2	5	6	2	5	3	6	3	3	6	2
Chipping Sparrow	6	3	6	5	4	6	4	6	5	3	4	4	4	5	6	5	5	6	4
White-crowned Sparrow	11	1	1	0	0	P	0	2	0	3	3	0	3	2	0	0	2	P	0
Subtotals		52	44	53	46	42	48	64	38	39	58	27	39	64	58	47	60	53	43
Totals		149			136			141			124			169			156		

a P = species that occurred on a plot in insufficient numbers for pair determination; 0 = species that did not occur on a plot; + = at least one observation suggested an additional pair, but not enough to call it a full breeding pair.

b of 180 possible instances of occurrance (9 plots x 20 species), 10 species (none ranked lower than 8th) were absent from 1-3 plots in 16 instances.

c rank in decreasing order of abundance from total pair estimates in all plots before treatment.

d likely includes E. traillii (Willow Flycatcher), E. hammondii (Hammond's Flycatcher), and E. oberholseri (Dusky Flycatcher); the latter was the most common.

Table 6. Estimated numbers of selected resident breeding pairs in each group of three plots with differing treatments and grouped by feeding strategy[a].

Feeding Strategy	No. Species	Control		Dylox		Sevin-4-oil	
		Pre	Post	Pre	Post	Pre	Post
Aerial	2	22	16	21	16	22	21
Tree-canopy	6	57	48	50	39	67	59
Tree-trunk	1	3	4	3	1	2	2
Ground	6	51	47	44	44	55	53
Understory	4	15	20	21	23	22	21
Air-ground	1	1	1	2	1	1	0
Total	20	149	136	141	124	169	156

[a]All species included from Table 5; see Table 1 for feeding strategies; species and their feeding strategies not included on Table 1 but used here are: Hermit Thrush - ground, Ruby-crowned Kinglet - tree-canopy, Lazuli Bunting - understory, and Cassins's Finch - tree-canopy feeders.

Table 7. Results of searching for sick or dead birds in untreated and treated major plots.

Treatment	Prespray			Postspray		
	Search Days[a]	Search Hours	No. Birds Found Dead	Search Days[a]	Search Hours	No. Birds Found Dead
Control	10	11.0	0	8	7.5	0
Dylox	10	13.0	1[b]	8	8.5	2[c]
Sevin-4-oil	12	13.5	4[d]	7	9.0	0

[a]Each search day represents one person searching in one plot for one day, for 0.5 to 2.5 hours.

[b]Blue Grouse.

[c]Newly hatched young found partly ingested by garter snakes.

[d]One each of adult Yellow-rumped Warbler, Blue Grouse, Common Flicker, and Western Tanager.

Table 8. Scientific names of birds mentioned in this paper (A.O.U. 1957, 1973).

Common Name	Scientific Name
Goshawk	*Accipiter gentilis*
Sharp-shinned Hawk	*Accipiter striatus*
Red-tailed Hawk	*Buteo jamaicensis*
Common Flicker	*Colaptes auratus*
Yellow-bellied Sapsucker	*Sphyrapicus varius*
Williamson's Sapsucker	*Sphyrapicus thyoideus*
Hairy Woodpecker	*Dendrocopos villosus*
Downy Woodpecker	*Dendrocopos pubescens*
Northern Three-toed Woodpecker	*Picoides tridactylus*
Flycatcher	*Empidonax* spp.
Tree Swallow	*Iridoprocne bicolor*
Mountain Chickadee	*Parus gambeli*
Black-capped Chickadee	*Parus atricapillus*
House Wren	*Troglodytes aedon*
American Robin	*Turdus migratorius*
Hermit Thrush	*Catharus guttatus*
Swainson's Thrush	*Catharus ustulatus*
Mountain Bluebird	*Sialia currucoides*
Ruby-crowned Kinglet	*Regulus calendula*
Warbling Vireo	*Vireo gilvus*
Yellow Warbler	*Dendroica petechia*
Yellow-rumped Warbler	*Dendroica coronata*
MacGillivray's Warbler	*Oporornis tolmiei*
Pine Siskin	*Spinus pinus*
Western Tanager	*Piranga ludoviciana*
Lazuli Bunting	*Passerina amoena*
Green-tailed Towhee	*Chlorura chlorura*
Cassin's Finch	*Carpodacus cassinii*
Dark-eyed Junco	*Junco hyemalis*
Chipping Sparrow	*Spizella passerina*
White-crowned Sparrow	*Zonotrichia leucophrys*

APPENDIX F

SPRUCE BUDWORM PILOT TEST OF TRICHLORFON (DYLOX) AND CARBARYL (SEVIN-4-OIL): II. The impact on brain cholinesterase activity in birds[1]

Joseph G. Zinkl,[2] Charles J. Henny,[2] and Lawrence R. DeWeese[3]

INTRODUCTION

Our study in the Beaverhead National Forest of southwestern Montana of the impact of trichlorfon (Dylox)[4] and carbaryl (Sevin-4-oil)[4] on resident breeding bird populations was outlined in the first report of this series (DeWeese and Henny 1976). The study plan included: (1) an evaluation of reproductive performance (nesting success), (2) the estimation of breeding pair density before and after spray within major habitats, (3) estimation of total birds at fixed stations in each major habitat, an approach distinct from the breeding pair estimates, (4) exploration into the food habits of the resident birds as they related to the spruce budworm and other important insect groups, and (5) determination

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[1]Results incomplete and not for publication or use without authority of the Director, Denver Wildlife Research Center.

[2]U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Bldg. 16, Federal Center, Denver, Colorado 80225.

[3]U.S. Fish and Wildlife Service, Denver Wildlife Research Center, P. O. Box C, Davis, California 95616.

[4]Reference to trade names does not imply U.S. Government endorsement of commercial products.

of brain cholinesterase activities from abundant and diverse avian species. The latter is the topic of this report.

Details of the plot locations, dates sprayed, application rates, formulation of the insecticides and operational summaries are not given here. Briefly, among nine 1-2,000-acre plots, three were sprayed by helicopter with a Sevin-4-oil formulation, three with Dylox and three were untreated. Each was a single application made early in the morning at a calculated rate of 1 lb/acre (active ingredient).

Since Dylox and Sevin-4-oil are organophosphate and carbamate insecticides, respectively, they inhibit cholinesterase enzymes. By specifically inhibiting acetylcholinesterase, they interfere with cholinergic nerve transmissions. Signs of cholinesterase inhibitor poisoning include myosis, salivation, and lacrimation (muscurinic effects) and muscle twitching, paralysis and clonic convulsions (nicotinic effects). Death is due to asphyxiation from paralysis of respiratory muscles and/or inhibition of the central respiratory center (O'Brien 1967:56).

Since cholinesterase activity is easily measured, its measurement can be used to determine if an animal has been poisoned with organophosphate or carbamate insecticides (Stickel 1974). However, certain precautions must be taken in order to assure that the results are valid. The first is that the cholinesterase activity of birds suspected to have been poisoned with cholinesterase inhibitors must be compared with that of unpoisoned birds of the same species because of the great variation of activity between species (Stickel 1974). The second is that

storage of the enzyme-containing tissue should be such as not to cause any deterioration of enzyme between the time of death and the time of analysis (Stickel 1974, Ludke et al. 1975). With these precautions in mind, brain cholinesterase activities were determined in birds collected from Montana forest areas sprayed with either Dylox or Sevin-4-oil.

MATERIALS AND METHODS

Birds were collected using mist nets or by shooting with shotguns. The birds collected with mist nets were killed by asphyxiation in CO_2. Either whole birds or heads were frozen on dry ice until the brains were dissected for analysis. Occasionally, the brain of a shot bird was discarded because of excessive damage. This precaution was taken because different areas of the brain have different cholinesterase activities (Knittle and Tucker 1974).

Control birds and treatment birds (spray area) were collected from similar habitats. Control birds were collected before spraying and during the time of spraying in order to determine if a short-term temporal change in cholinesterase activity occurred. Since both sexes were collected, it was also possible to determine if there were any differences due to sex.

The Ennis High School science laboratory was kindly donated for laboratory space. All analyses were carried out at this location within 12 hours after collection.

After removal from the calvarium, brains were homogenized in cold 0.1 M phosphate buffer (pH 7.4) at a 1-5 dilution. They were then diluted to either 1-50 or 1-100 with the phosphate buffer just prior to analysis.

The Ellman (Ellman et al. 1961) method was adapted to determine brain cholinesterase activity (Dieter and Ludke 1975). The reagents for this technique were obtained in kit form from BMC Corporation, Dallas, Texas. A Spectronic 88 (Bausch & Lomb) fitted with a flow-through, water-jacketed curvette was used for determining the activity. Optical density readings were taken every 30 seconds for 3 minutes in order to assure that the reaction was linear. All analyses were carried out at 25°C.

RESULTS

Cholinesterase activities of 27 species of the orders Passiformes (24 species) and Piciformes (3 species) were determined. However, for several species insufficient data were obtained to be useful in evaluating the effects of the spray. No short-term temporal effects or sex differences were found. The species with the highest activities were the yellow-bellied sapsucker (Sphyrapicus varius) and the hairy woodpecker (Dendrocopos villosus) (47.2 and 42.5 mU/mg brain, respectively).

Sufficient data were obtained from 10 species of birds to evaluate the effect of Dylox on brain cholinesterase activity. One dark-eyed junco (Junco hyemalis), one evening grosbeak (Hesperiphona vespertina), two mountain chickadees (Parus gambeli) and two western tanagers (Piranga ludoviciana) had values which were at least 2 standard deviations (S.D.) below the mean (Table 1). Both western tanagers' activities were more than 20% below the mean (26.5% and 20.5%) while the evening grosbeak's activity was depressed nearly to that level (19.8%). These

western tanagers were collected on the day of spray, while the evening grosbeak was collected 3 days after the spraying.

Of the 12 species of birds evaluated from the Sevin-4-oil spray areas, 3 individuals representing 3 species had values depressed greater than 2 S.D. below the mean (Table 2). They were a mountain chickadee, an evening grosbeak and a Lincoln's sparrow (Melospiza lincolnii). Only the evening grosbeak's brain cholinesterase activity was more than 20% below the mean (21.3%). This evening grosbeak was collected on the day of spray.

DISCUSSION

Previous work in our laboratory with starling (Sturnus vulgaris) brains and sera showed that storage in dry ice preserves cholinesterase enzyme activity for up to 5 weeks (Zinkl and Hudson 1975). Knittle and Tucker (1974) have shown that storage at -40°C and -68°C preserves the enzyme. However, deterioration does occur at -18°C (Knittle and Tucker 1974) or -22°C (Ludke et al. 1975). In this study it is unlikely that there was any loss of activity from the time of collection until analysis because the brains were stored in dry ice and the analyses were carried out soon after collection (within 12 hours).

A considerable difference of opinion exists among authors regarding how great the brain cholinesterase depression must be for diagnosing cause of death. Ludke et al. (1975) showed that 50% inhibition occurred in Japanese quail (Coturnix c. japonica) that died after being fed up to 1,400 ppm parathion for up to 5 days. Bunyan et al. (1968) found that pheasants dying from a single dose of a variety of organophosphates had

at least 90% brain cholinesterase depression. In our laboratory, ring doves given a single dose of 21.2 mg Dylox/kg B.W. had 83% brain cholinesterase depression when sacrificed 2 hours after dosing. Others given this amount survived. Ring doves that died after being given 42.4 mg Dylox/kg B.W. had 95% depression. Homing pigeons given 195 mg Dylox/kg B.W. died within 45 minutes after dosing. Their brain cholinesterase activities were 83% inhibited. Others given 78.1 mg Dylox/kg B.W. survived for 18 hours before being sacrificed. Their activities were depressed 68% at that time even though they were showing few signs of organophosphate toxicity. Ring doves given 1,000 mg Sevin-4-oil/kg B.W. had brain cholinesterase activities that were decreased 56% when sacrificed 2 hours after dosing. Other birds given the same dose survived (Zinkl and Hudson 1975).

Therefore, even using the most stringent criteria (50% depression), no birds were in immediate danger of dying from either Dylox or Sevin-4-oil poisoning. However, at least 4 of the birds had activities depressed about 20% below the mean of the species. This indicates exposure had occurred (Ludke et al. 1975). Five more birds had activities depressed greater than 2 S.D. below the mean. Of the 5 species having depressed activities ($\bar{x}$ - 2 S.D.) 3 are canopy dwellers (mountain chickadee, evening grosbeak, and western tanager). These birds represented 7 of the 9 depressed values, and they were the most depressed values, probably reflecting greater exposure of these species rather than increased susceptibility to the chemicals.

Most of the depressed values occurred on the day of spray (day 0), probably due to the transient environmental nature of the compounds

(especially Dylox) (Kaemmerer and Buntenbotter 1973:201, Paris and Lewis 1973).

There is no experimental work concerning the effects of sublethal cholinesterase inhibition on birds. Perhaps these levels might increase a bird's susceptibility to predation or decrease its ability to obtain food (e.g., fly-catching). Nevertheless, they represent a small number of birds compared to the total evaluated.

In conclusion, spraying with Dylox or Sevin-4-oil at 1 lb/acre (active ingredient) had little effect on brain cholinesterase activities. Thus, only minimal exposure occurred, a finding similar to that of Kurtz and Studholme (1974) who determined residues in birds from eastern forests sprayed with Dylox and Sevin.

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Table 1. Brain Cholinesterase Activities[1] of Birds Taken From Areas Sprayed With Dylox

Species	Control[2]	Normal Range ($\bar{x} \pm 2$ S.D.)	Day 0[3]	Day 1	Day 2	Day 3	Day 5	Abnormally Low Values
Empidonax spp.[4]	22.2±2.6 (8)	17.0 - 27.4	24.6±0.5 (2)	-	-	-	-	
Evening Grosbeak (Hesperiphona vespertina)	31.9±2.1 (6)	27.7 - 36.1	33.1 (1)	30.9 (1)	30.3 (1)	28.1±3.5 (2)	30.4±2.8 (3)	25.6 (day 3)
Pine Siskin (Spinus pinus)	22.2±2.4 (10)	17.4 - 27.0	25.8±1.1 (2)	-	-	22.0 (1)	25.5±1.5 (3)	
Chipping Sparrow (Spizella passerina)	23.5±1.8 (7)	19.9 - 27.1	24.5±1.3 (4)	24.4±3.5 (2)	23.4±1.1 (3)	24.7±1.9 (3)	24.2±1.4 (5)	
Dark-eyed Junco (Junco hyemalis)	33.2±1.1 (9)	31.0 - 35.4	35.6±1.8 (8)	36.7±1.4 (3)	32.3±1.0 (4)	35.6±1.7 (6)	35.6±2.5 (3)	30.9 (day 2)
Lazuli Bunting (Passerina amoena)	31.6±2.7 (5)	26.2 - 37.0	31.0 (1)	-	-	32.0 (1)	-	
Western Tanager (Piranga ludoviciana)	28.3±2.9 (8)	22.5 - 34.1	21.7±1.2 (2)	30.0±2.3 (3)	30.1±1.6 (4)	30.2±1.3 (3)	29.7 (1)	22.5 (day 0) 20.8 (day 0)

Table 1 (cont'd)

Warbling Vireo	33.8±3.9	26.0 - 41.6	35.0±1.5	-	29.3	31.6	-		
(Vireo gilvus)	(10)		(3)		(1)	(1)			
Mountain Chickadee	33.8±1.3	31.2 - 36.4	30.9±3.2	34.6±2.9	32.3±2.0	31.4	33.2±0.6	28.6	30.9
(Parus gambeli)	(3)		(2)	(4)	(2)	(1)	(3)	(day 0)	(day 2)
American Robin	26.6±3.9	18.8 - 34.4	29.6±3.4	28.1±2.2	28.0	29.7±0.3	31.5±2.6		
(Turdus migratorius)	(10)		(3)	(3)	(1)	(3)	(4)		

Activities expressed as mU/mg brain.

Mean and standard deviation.

Days after spraying.

Empidonax spp. were primarily dusky flycatchers (Empidonax oberholseri).

Table 2. Brain Cholinesterase Activities[1] of Birds Taken From Areas Sprayed With Sevin-4-oil

Species	Control[2]	Normal Range ($\bar{x} \pm 2$ S.D.)	Day 0[3]	Day 1	Day 2	Day 5	Abnormally Low Values
Yellow-bellied Sapsucker	47.2±4.3	38.6 - 55.8	50.7±2.6	-	-	-	
(*Sphyrapicus varius*)	(4)		(2)				
Common Flicker	24.8±1.2	22.4 - 27.2	25.7±0.5	-	-	-	
(*Colaptes auratus*)	(3)		(2)				
Empidonax spp.[4]	22.2±2.6	17.0 - 27.4	24.2±1.0	-	-	28.4	
	(8)		(4)			(1)	
Evening Grosbeak	31.9±2.1	27.7 - 36.1	27.7±2.3	31.2±2.3	-	32.4±2.4	25.1
(*Hesperiphona vespertina*)	(6)		(3)	(2)		(4)	(day 0)
Pine Siskin	22.2±2.4	17.4 - 27.0	22.6±1.3	23.0	-	22.1±0.9	
(*Spinus pinus*)	(10)		(7)	(1)		(3)	
Chipping Sparrow	23.5±1.8	19.9 - 27.1	24.2±1.6	-	-	26.4±0.5	
(*Spizella passerina*)	(7)		(5)			(3)	
Dark-eyed Junco	33.2±1.1	31.0 - 35.4	33.5±1.1	-	31.9	36.2±0.8	
(*Junco hyemalis*)	(9)		(6)		(1)	(4)	
Lincoln's Sparrow	25.2±1.4	22.4 - 28.0	24.1±2.5	24.9	-	24.8±0.7	21.6
(*Melospiza lincolnii*)	(5)		(4)	(1)		(2)	(day 0)

able 2 (cont'd)

estern Tanager	28.3±2.9	22.5 - 34.1	29.8±2.5	-	-	-	
(Piranga ludoviciana)	(8)		(6)				
arbling Vireo	33.8±3.9	26.0 - 41.6	32.7±2.6	-	-	33.0	
(Vireo gilvus)	(10)		(4)			(1)	
ountain Chickadee	33.8±1.3	31.2 - 36.4	32.6±0.8	-	30.9±1.2	-	29.5
(Parus gambeli)	(3)		(3)		(3)		(day 2)
erican Robin	26.6±3.9	18.8 - 34.4	28.9±2.9	29.0±0.4	28.8±1.5	30.5±1.9	
(Turdus migratorius)	(10)		(6)	(4)	(3)	(5)	

ctivities expressed as mU/mg brain.

ean and standard deviation.

ays after spraying.

mpidonax spp. were primarily dusky flycatchers (Empidonax oberholseri).

APPENDIX G: Aquatic monitoring, 1975 Beaverhead National Forest Dylox test.--Monitoring was conducted in two streams within spray blocks treated with Dylox on the Beaverhead National Forest in July 1975. Streams monitored were the South Fork Warm Springs and Warm Springs Creeks. Data from the project is presently being analyzed. Preliminary analyses indicate that Dylox caused a measurable increase in drift of mayflies (Ephemeroptera), stoneflies (Plecoptera), and true flies (Diptera) one-half to 2 hours after application of Dylox to the South Fork Warm Springs drainage treatment block. On main Warm Springs Creek, no increase in drift after application was measured. Trout placed in live cages in the streams 1 to 2 weeks prior to treatment and removed for residue analysis showed no physical disability as a result of the application. Residue analyses in fish tissue were reported at 0.03 ppm. Aquatic insects taken from the treatment streams 80 hours after application had residue levels of 0.50 to 0.96 ppm. Streams had a pH of 8.8 and alkaline levels of 102 ppm (South Fork Warm Springs Creek) and 136 ppm (Warm Springs Creek). Substrate sampling in October showed a good number and species diversity of aquatic insects in both streams (Personal communication from G. Haugen (1976, Fisheries Biologist, Gallatin National Forest).

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